change in stroma which usually occurs in invasive lesion (Fig. 1) [5]. When a tumor had areas of tumor nests surrounded by thin fibrous septa without elastic fibers, the areas were excluded from ASF area. We determined the ASF ratio by visual examination using H&E and EvG staining specimens and 70% as a threshold (data not shown). A tumor nest was defined as either a nested group of tumor cells or a single tumor cell invading individually, similarly the MTN was defined as the smallest group of tumor cells observed in the primary tumor. The size of the MTN in each patient was classified as follows according to the report of Maeshima et al.: large nest (consisting of more than 6 tumor cells), small nest (consisting of 2–5 tumor cells) or single cell [6]. When discordant assessments arose, consensus was achieved after joint review of the specimen over a multiheaded microscope.

2.3. Statistical analysis

Log-rank test was used to select significant factors examined for discriminating the patients with a relapse from those without a relapse. To identify which factors had a significant influence on the patients' outcome, logistic regression models were used. The correlations between clinicopathological variables were evaluated by the chi-square test or Fisher's exact test, as appropriate. *P*-values <0.05 were considered significant.

3. Results

We reviewed 81 patients with SqCC of the peripheral lung measuring 30 mm or less in diameter. Clinicopathological characteristics are shown in Table 1. Peripheral SqCC patients showed male preponderance (89%) and high smoking habit (99%). In the present population, 70% took lobectomy, 80% diagnosed as T1a or T1b, 75% showed the tumor size over 20 mm, vessel invasion was more frequent than lymphatic permeation and lymph node metastasis was only 9%. UIP was observed in 9% of the patients. The recurrence rate for all patients was 19.7%. In the univariate analysis, only two factors had a statistically significant influence on the patients' outcome (Table 2). Neither the patients with ASF ratio 70% or more nor the maximum diameter of invasive area of 10 mm or less in size relapsed (ASF area, $P=0.0214$; maximum diameter of invasive area, $P=0.0373$). None of other factors; MTN size, lymph node metastasis, vessel invasion and lymphatic permeation showed significant difference in disease free survival (DFS) rate. Multivariate analysis showed that the ASF ratio 70% or more ($P=0.0337$), not the maximum diameter of invasive area ($P=0.2136$), significantly affected the outcome of the patients (Table 3). Fig. 2 shows disease-free survival curves for patients with ASF ratio 70% or more and ASF ratio less than 70%. Table 4 shows the clinicopathological characteristics of the tumors with ASF ratio 70% or more in comparison to those with ASF ratio less than 70%. The tumors with ASF ratio 70% or more showed female preference, less maximum diameter of tumor invasion size or vascular invasion, less single cell pattern, and less inflammation. Moreover, no patients with those tumors showed recurrence or death.

Table 1
Patients characteristics ($n=81$).

	Number of patients (%)
Total	81
Gender	
Male	72 (89%)
Female	9 (11%)
Age (years, range)	70.5 (47–84)
Smoking habit	
Non-smoker	1 (1%)
Past-smoker	36 (45%)
Current-smoker	44 (54%)
Surgery	
Lobectomy + LN	57 (70%)
Partial resection	24 (30%)
T factor	
T1a	22 (27%)
T1b	43 (53%)
T2	16 (20%)
Tumor size	
<20 mm	20 (25%)
20–30 mm	61 (75%)
Lymph node metastasis	
Negative	74 (91%)
Positive	7 (9%)
Vessel invasion	
Negative	55 (68%)
Positive	26 (32%)
Lymphatic permeation	
Negative	71 (87%)
Positive	10 (13%)
Pleural involvement	
P0	65 (80%)
P1–3	16 (20%)
Exposure to asbestos	
Negative	72 (88%)
Positive	9 (12%)
Tumor location	
Patients without UIP	
Upper lobe	39 (48%)
Middle and lower lobe	33 (41%)
Patients with UIP	
Upper lobe	2 (2%)
Middle and lower lobe	7 (9%)

SqCC, squamous cell carcinoma; UIP, usual interstitial pneumonia.

4. Discussion

In the present study, we evaluated several clinicopathological factors to reveal prognostic indications for peripheral lung SqCCs and found ASF ratio to be significant. The multivariate analysis showed that the patients having focally invasive tumors with ASF ratio 70% or more had no recurrence as same as those pure ASF type SqCCs (ASF ratio 100%).

The definition of ASF depends on the observation of alveolar EFF whose complete preservation is considered to represent noninvasive area. It has been well-known in lung adenocarcinoma that the preservation of alveolar structure [10–13] and alveolar EFF has significant association with prognosis [14–16]. Funai et al. considered pure ASF type tumors on peripheral SqCCs of the lung to be non-invasive cancers which were thought to be equivalent to tumors without EFF destruction on adenocarcinomas of the lung [5]. However, only 5% (5 cases of 109) of the patients had pure ASF type. That was only 1 case 1.2% in our study. The prognostic factors that are applicable to the majority of peripheral SqCCs have been desired. Since Funai et al. did not refer the proportion of ASF, we attempted to evaluate the ratio to clarify the significance as a prognostic factor. In the present study, nineteen patients (23.5%) had a tumor with ASF ratio70% or more which was equivalent to a pure ASF type tumor of the Funai's study; we were able to select more cases with favorable prognosis in manner of detailed analysis of ASF ratio than Funai's study. In addition, the invasive tumors with ASF ratio 70% or more may appear to have low grade malignancy and no recurrence, therefore it could be said that they are micro-invasive carcinomas.

In 2009, Samuel and Yousem studied 62 peripheral SqCCs of lung focusing on the patterns of marginal or peripheral growth and rate of an alveolar filling (AF) component [17]. Comparing those tumors with 20% or more of their diameters composed of an AF pattern to those tumors of equivalent diameter without AF pattern, no differences in average survival were observed. In our advance investigation, we obtained the same result (data was not shown).

Table 2
Examination of prognostic factors on recurrence in peripheral squamous cell carcinoma of lung by univariate analysis.

Clinicopathological factors	Number of no recurrence	Number of recurrence	Univariate analysis (*P* value)
Total	65	16	
Gender			
Male	57	15	0.676
Female	8	1	
Brinkman index			
0–999	34	8	0.870
1000–	31	8	
Smoking habit			
Current smoker	8	36	0.765
Past smoker	8	28	
No smoker	0	1	
T factor			
T1a	17	4	0.792
T1b	36	8	
T2	12	4	
Lymph node metastasis			
Negative	74	16	0.229
Positive	7	0	
Vessel invasion			
Negative	44	11	0.701
Positive	21	5	
Lymphatic permeation			
Negative	55	16	0.146
Positive	10	0	
Necrotising area			
<50%	47	12	0.869
≥50%	18	4	
Pleural involvement			
P0	53	12	0.541
P1–3	12	4	
Size of minimal tumor nest			
Large nest	4	0	0.284
Small nest	15	2	
Single cell	46	14	
Differentiation			
Well	5	3	0.314
Moderate	27	7	
Poor	33	6	
Alveolar space filling			
≥70%	19	0	0.0214
<70%	46	16	
Inflammatory cell infiltration			
Mild	60	11	0.304
Moderate or severe	21	5	
Background lung			
With UIP	7	2	0.739
Without UIP	58	14	
Diameter of invasion area			
<10 mm	16	0	0.0373
≥10 mm	49	16	

The maximum diameter of invasive area (MDIA) of 10 mm or less in size had a statistically significant influence on the patients' outcome in univariate analysis but not in multivariate analysis. MDIA in peripheral SqCC had not been well evaluated, although the MDIA of 5 mm or less in adenocarcinoma was proved to be a good prognosis factor and thought to represent a microinvasive carcinoma [18]. We think that MDIA lost significance because ASF ratio 70% or more became a confounding factor to MDIA as shown in Table 4.

Table 3
Examination of prognostic factors on recurrence in peripheral squamous cell carcinoma of lung by multivariate analysis.

Parameters	*P* value
ASF ratio 70% or more	0.038
Invasive diameter 10mm or less	0.094
T factor	0.454
Age	0.428
Histological differentiation	0.493
Size of minimal tumor nest	0.826
Sex	0.936

Table 4
Correlation between clinicopathological features and peripheral squamous cell carcinoma of lung with ASF ratio 70% or more and less than 70%.

Factors	ASF ≥ 70% (*n* = 19)	70% > ASF (*n* = 62)	*P* value
Sex			0.046*
Male	14	58	
Female	5	4	0.702
Age (years)			
<70	8	21	
≤70	11	41	
Asbestos exposure			0.745
Absent	16	56	
Present	3	6	
Tumor size (mm)			0.221
≤20	8	15	
<20	11	47	
T			0.867
1a + 1b	16	49	
2	3	13	
N			0.894
0	18	56	
1 or 2	1	6	
ly			0.357
Negative	15	56	
Positive	4	6	
v			0.043*
Negative	17	38	
Positive	2	24	
PL			0.867
P0	16	49	
P1–3	3	13	
pm			0.528
pm0	19	61	
pm1	0	1	
Differentiation		0.226	
Well	0	8	
Moderate or poorly	19	54	
Necrosis			0.696
Mild	15	44	
Moderate or severe	4	18	
Nesting			0.020*
Small or large	10	51	
Single cell	9	11	
Tumor invasion (mm)			0.00*
≤10	12	4	
<10	7	58	
Inflammation			0.040*
Mild	18	42	
Moderate or severe	1	20	
Interstitial pneumonia			0.178
Absent	19	53	
Present	0	9	
Recurrence			0.032*
Absent	19	46	
Present	0	16	
Death			0.032*
Live	19	46	
Died	0	16	

* Statistically significant factor.

Inflammation is generally thought to be a positive prognostic factor in non-small cell carcinoma, but not in this case [19]. We have supposed that previous reports which regarded inflammation as a favorable factor conducted prognostification in patients with advanced cancers. But we compared probably minimally invasive cancers with overt invasive cancers; therefore analysis of inflammation might bring an opposite result.

In other studies, vascular invasion, lymphatic permeation, and perineural invasion have been reported to be important prognostic factors in peripheral SqCC. Saijo et al. reported that peripheral type SqCC had higher vascular invasion and higher pleural invasion and

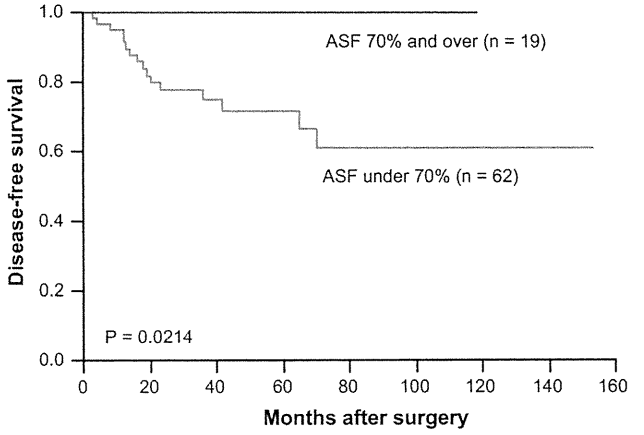

Fig. 2. Disease-free survival curves for patients with ASF ratio 70% or more and ASF ratio less than 70%.

with less lymph node metastasis compared with central type SqCC [20]. In our study those all factors had not influence to patient's outcome, however, only vascular invasion was observed frequently in the tumors with ASF ratio less than 70%, significantly, and probably the study of Saijo et al. included many invasive tumors with ASF less than 70%.

We propose from the present data that peripheral invasive SqCC with ASF ratio 70% or more could be classified as microinvasive carcinoma and peripheral SqCC with ASF ratio 100% as noninvasive carcinoma. Anatomical lobectomy with mediastinal lymph node dissection is the present standard of care for such patients with stage I or II, and adjuvant chemotherapy may be performed in patients with stage IB or more advanced stage. In a similar manner to noninvasive adenocarcinoma of the lung, limited resection could be an acceptable treatment for a tumor with ASF ratio 100% and partial resection or simple lobectomy without lymph node dissection and/or adjuvant therapy for a tumor with ASF ratio 70% or more in the future. However, since it might be difficult to select candidate cases of favorable peripheral SqCC by the radiological procedure in the present, additional studies are needed before modification of the standard therapy by the basis of the proposed classification.

Conflict of interest statement

The authors report no conflicts of interest.

Acknowledgements

We thank Ms. Seki and Ms. Ueda for their technical assistance. This work was supported in part by the Kanagawa Cancer Research Fund and Kanagawa Prefectural Hospitals Cancer Research Fund.

References

[1] Kakizoe T, Sugiura S, Segami K, Yamaguchi N, Shimizu H, Yamada T, et al. Cancer statistics in Japan 1999. Tokyo, Japan: Foundation of Promotion of Cancer Research; 1999. p. 12.
[2] Travis WD, Brambilla E, Muller-Hermelink HK, Harris CC, editors. World Health Organization classification of tumors. Pathology and genetic of tumors of the lung, pleura, thymus and heart. Lyon: IARC Press; 2004. p. 26–30.
[3] Furonaka O, Takeshima Y, Awaya H, Ishida H, Kohno N, Inai K. Aberrant methylation of *p14ARF*, *p15INK4b* and *p16INK4a* genes and location of the primary site in pulmonary squamous cell carcinoma. Pathol Int 2004;54: 549–55.
[4] Sakurai H, Asamura H, Watanabe S, Suzuki K, Tsuchiya R. Clinicopathologic features of peripheral squamous cell carcinoma of the lung. Ann Thorac Surg 2004;78:222–7.
[5] Funai K, Yokose T, Ishii G, Araki K, Yoshida J, Nishimura M, et al. Clinicopathological characteristics of peripheral squamous cell carcinoma of the lung. Am J Surg Pathol 2003;27:978–84.
[6] Maeshima A, Maeshima A, Asamura H, Matsuno Y. Histologic prognostic factors for small-sized squamous cell carcinomas of the peripheral lung. Lung Cancer 2006;52:53–8.
[7] Carter D. Squamous cell carcinoma of the lung: an update. Semin Diagn Pathol 1985;2(4):226–34.
[8] Sobin L, Gospodarowicz M, Wittekind C, editors. International union against cancer. TNM classification of malignant tumours. 7th ed. West Sussex, UK: John Wiley and Sons Ltd.; 2009.
[9] American Thoracic Society, European Respiratory Society. American Thoracic Society/European Respiratory Society international multidisciplinary consensus classification of idiopathic interstitial pneumonias. General principles and recommendations. Am J Respir Crit Care Med 2002;165:277–304.
[10] Kurokawa T, Matsuno Y, Noguchi M, Mizuno S, Shimosato Y. Surgically curable early adenocarcinoma in the periphery of the lung. Am J Surg Pathol 1994;18(5):431–8.
[11] Terasaki H, Niki T, Matsuno Y, Yamada T, Maeshima A, Asamura H, et al. Lung adenocarcinoma with mixed bronchioalveolar and invasive components. Clinicopathological features, subclassification by extent of invasive foci, and immunohistrochemical characterization. Am J Surg Pathol 2003;27:937–51.
[12] Fujita A, Kameda Y, Goya T. Clinicopathology of stromal invasion in lung adenocarcinoma. Pathol Int 2009;59:1–6.
[13] Sakuma Y, Okammoto N, Saito H, Yamada K, Yokose T, Kiyohashi M, et al. A logistic regression predictive model and the outcome of patients with resected lung adenocarcinoma of 2 cm or less in size. Lung Cancer 2009;65: 85–90.
[14] Noguchi M, Morikawa A, Kawasaki M, Matsuno Y, Yamada T, Hirohashi S, et al. Small adenocarcinoma of the lung. Histologic characteristics and prognosis. Cancer 1995;75(12):2844–52.
[15] Yokose T, Suzuki K, Nagai K, Nishikawa Y, Sasaki S, Ochiai A, et al. Favorable and unfavorable morphological prognostic factors in peripheral adenocarcinoma of the lung 3 cm or less in diameter. Lung Cancer 2000;29:179–88.
[16] Goto K, Yokose T, Kodama T, Nagai K, Nishikawa Y, Ando M, et al. Detection of early invasion on the basis of basement membrane destruction in small adenocarcinomas of the lung and its clinical implications. Mod Pathol 2001;5:274–84.
[17] Samuel A, Yousem. Peripheral squamous cell carcinoma of lung: patterns of growth with particular focus on airspace filling. Human Pathol 2009;40: 861–7.
[18] Sakurai H, Maeshima A, Watanabe S, Suzuki K, Tsuchiya R, Maeshima AM, et al. Grade of stromal invasion in small adenocarcinoma of the lung. Histopathological minimal invasion and prognosis. Am J Surg Pathol 2004;28:198–206.
[19] Ruffini E, Asioli S, Filosso PL, Lyberis P, Bruna MC, Macri L, et al. Clinical significance of tumor-infiltrating lymphocytes in lung neoplasms. Ann Thorac Surg 2009;87:371–2.
[20] Saijo T, Ishii G, Nagai K, Funai K, Nitadori J, Tsuta K, et al. Differences in clinicopathological and biological features between central-type and peripheral-type squamous cell carcinoma of the lung. Lung Cancer 2006;52:37–45.

Ohe et al. Diagnostic Pathology 2011, 6:92
http://www.diagnosticpathology.org/content/6/1/92

DIAGNOSTIC PATHOLOGY

CASE REPORT Open Access

Stromal micropapillary pattern predominant lung adenocarcinoma - a report of two cases

Miki Ohe[1,2,4*], Tomoyuki Yokose[1], Yuji Sakuma[3], Sachie Osanai[1], Chikako Hasegawa[1], Kota Washimi[1], Kimitoshi Nawa[2], Tetsukan Woo[2], Rurika Hamanaka[2], Haruhiko Nakayama[2], Yoichi Kameda[1], Kouzo Yamada[2] and Takeshi Isobe[4]

Abstract

Generally, adenocarcinomas with micropapillary pattern, featuring small papillary tufts lacking a central fibrovascular core, are thought to have poor prognosis. This pattern has been described in various organs. However, tumor cells with micropapillary pattern of lung adenocarcinoma are more often seen to float within alveolar spaces (aerogenous micropapillary pattern, AMP) than in fibrotic stroma like other organs (stromal micropapillary pattern, SMP) and SMP predominant lung adenocarcinoma (SMPPLA) has not been well described yet. We presented two cases of SMPPLA which were found in the last four years. Both the cases showed more than 50% of SMP in the tumor area. The majority of the stromal micropapillary clusters expressed MUC1 and epithelial membrane antigen along the outer surface of cell membrane. On the other hand, connective tissues surrounding stromal micropapillary clusters showed no reactivity for epithelial markers (thyroid transcription factor-1 and cytokeratin) or endothelial marker (D2-40 and CD34). It means clusters of SMP do not exist within air space or lymphatic or vessel lumens. The tumors with SMP often presented lymphatic permeation and vessel invasion, and intriguingly, one of the two cases showed metastasis to the mediastinal lymph node. Additionally, both the cases showed *EGFR* point mutations of exon 21. These results suggest that SMPPLA might be associated with poor prognosis and effective for EGFR tyrosine kinase inhibitors.

Keywords: lung adenocarcinoma, micropapillary subtype, stromal micropapillary pattern, aerogeneous micropapillary pattern

Background

A new lung adenocarcinoma classification has been proposed by the International Association for the Study of Lung Cancer, American Thoracic Society and European Respiratory Society (IASLC/ATS/ERS). In this classification, the micropapillary subtype of lung adenocarcinoma (MSLA) was recommended as a newly added subtype of lung adenocarcinoma to lepidic, acinar, papillary, and solid subtypes defined in the 2004 World Health Organization (WHO) classification [1,2]. Generally, the micropapillary pattern is defined as tumor cells growing in papillary tufts, which lack fibrovascular cores surrounded by lacunar spaces and has been reported to be associated with a high incidence of nodal metastasis and poor prognosis [3-6]. This pattern has been described in various organs such as breast [7,8], urinary bladder [9,10], ovary [11,12], salivary gland [13], and is known to behave aggressively. In other organs than the lung, this pattern was observed mainly in stroma as invasive components (stromal micropapillary pattern: SMP) [7-19]; however in lung, MSLA is widely recognized as floating tumor cells within alveolar spaces (aerogenous micropapillary pattern: AMP) [3,4].

We examined whether SMP predominant subtypes were present in lung adenocarcinoma. During the period from February 2007 to December 2010, 559 patients with lung adenocarcinoma were consecutively treated by surgical resection at the Kanagawa Cancer Center, Kanagawa, Japan, and we found only two cases of SMP predominant lung adenocarcinoma (SMPPLA) (0.36%). We reported the cases of SMPPLA and attempted to describe the clinicopathological features.

* Correspondence: ohem@kcch.jp
[1]Department of Pathology, Kanagawa Cancer Center, Kanagawa, Japan
Full list of author information is available at the end of the article

Case presentation

Clinical summary

Case1

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

Case2

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

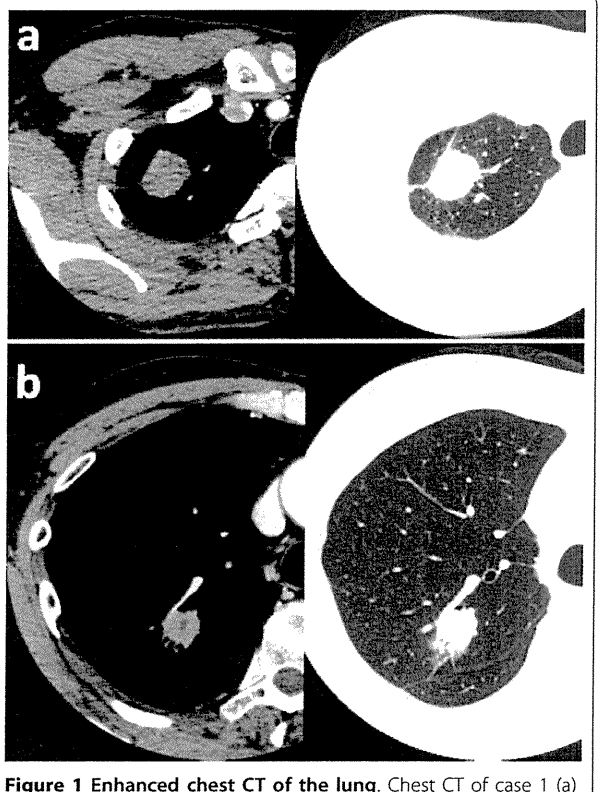

Figure 1 Enhanced chest CT of the lung. Chest CT of case 1 (a) and case 2 (b) showed a tumor of the right upper lobe of the lung. (a) Chest CT revealed a tumor with pleural indentation, without ground glass opacity (GGO). The tumor was 32 mm in diameter and mildly enhanced. (b) Chest CT revealed a nodule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

Pathological findings

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

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

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

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

Table 1 Antibodies used and immunohistchemical result

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

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

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

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

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

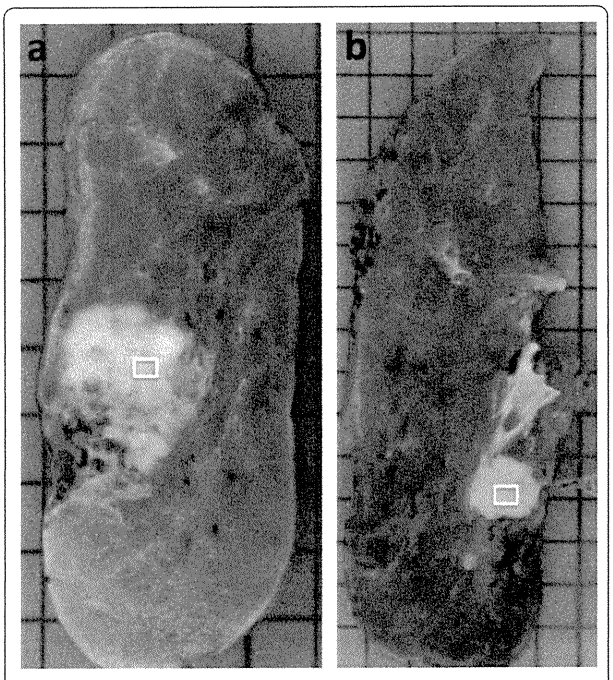

Figure 2 Macroscopic findings. Macroscopic examination of excised specimens showed the tumor measuring 36 × 25 × 30 mm in size, white in color on cut surface in the case 1 (a), and the tumor measuring 33 × 20 × 40 mm in size, grayish-white in color on cut surface containing areas of hemorrhage in the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

Mutational analysis

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

Discussion

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

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

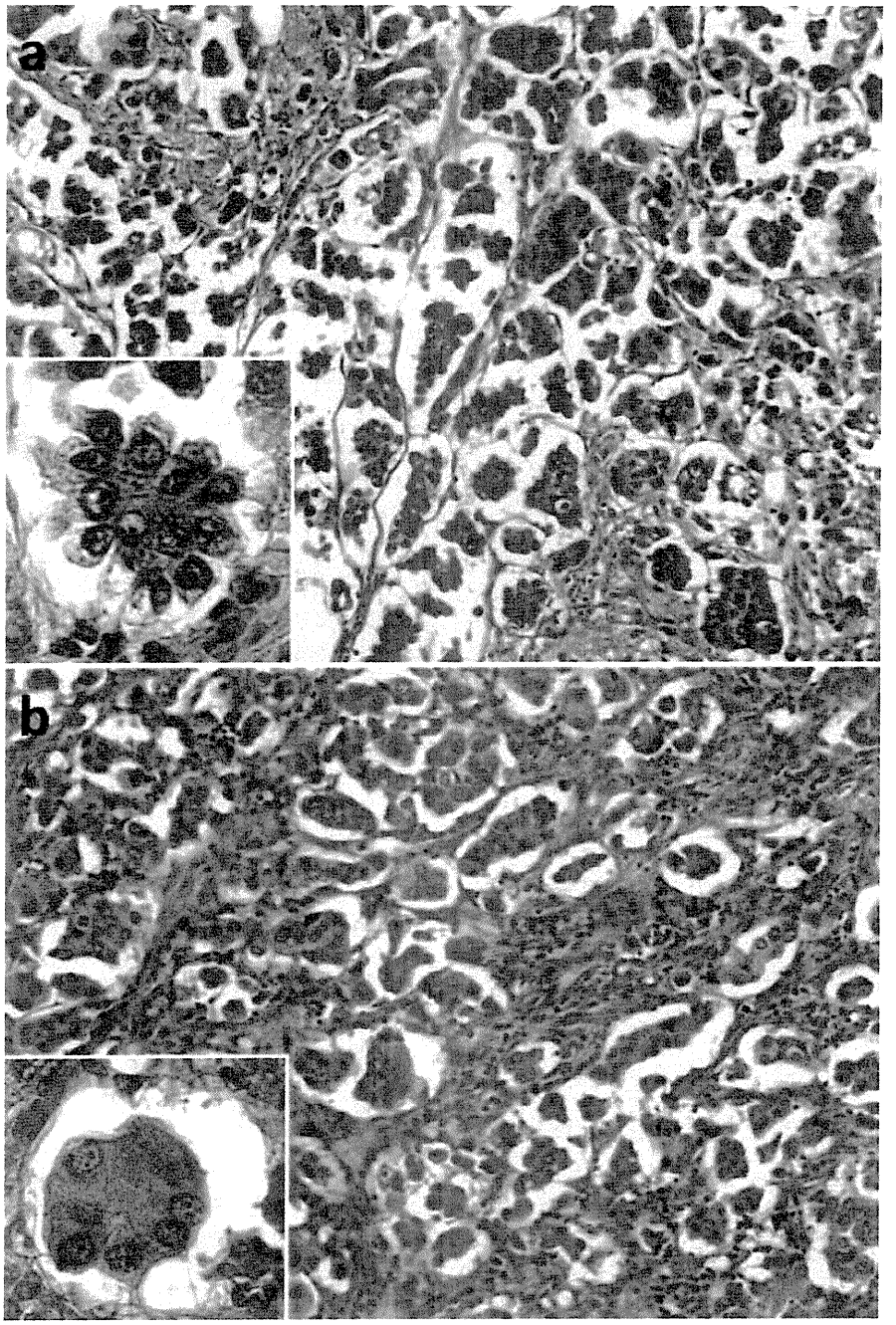

Figure 3 Photomicrographs. Stromal micropapillary pattern characterized by papillary structures with tufts lacking a central fibrovascular core and surrounding clear spaces in fibrotic stroma was seen in both case 1 (a) and case 2 (b). (HE stains ×100; inset, ×400)

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

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

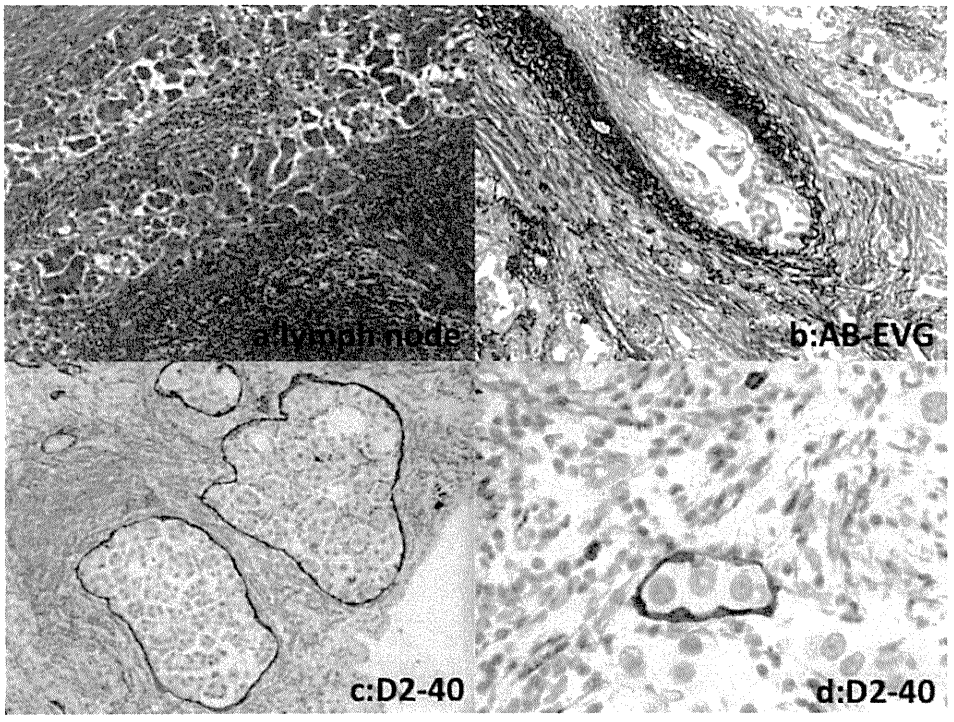

Figure 4 Photomicrographs. (a) A resected regional lymph node had metastatic foci composed of tumor cells with a micropapillary pattern in the case 1. (HE stain, ×200) (b) Vessel invasion by tumor cells in the case 1. (AB-EVG stain, ×100) (c, d) Tumor cells in lymphatic ducts, which were covered by D2-40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

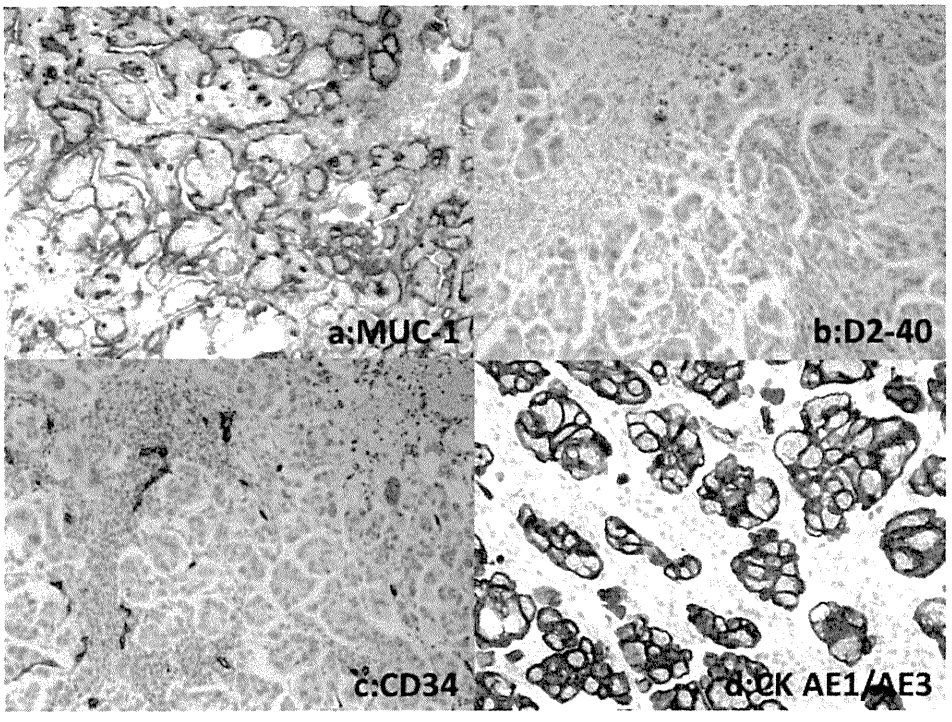

Figure 5 Immunohistochemical photomicrographs of case 1. (a) Tumor clusters of stromal micropapillary pattern had MUC1 expression on the outer surface strongly, indicating 'inside-out' pattern. (×200) (b) D2-40 and (c) CD34 were not found in cells consisting micropapillary tufts and (d) CK was not found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

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

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

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

Consent

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

Abbreviations

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

Acknowledgements

The authors thank the technicians of the Pathology Laboratory Division in the hospital for the technical assistance.

Author details

[1]Department of Pathology, Kanagawa Cancer Center, Kanagawa, Japan. [2]Department of Thoracic Oncology, Kanagawa Cancer Center, Kanagawa, Japan. [3]Molecular Pathology and Genetics Division, Kanagawa Cancer Center Research Institute, Kanagawa, Japan. [4]Division of Clinical Oncology and Respiratory Medicine, Department of Internal Medicine, Shimane University Faculty of Medicine, Shimane, Japan.

Authors' contributions

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

Competing interests

The authors declare that they have no competing interests.

Received: 1 July 2011 Accepted: 29 September 2011
Published: 29 September 2011

References

1. Travis WD, Brambilla E, Noguchi M, Nicholson AG, Geisinger KR, Yatabe Y, Beer DG, Powell CA, Riely GJ, Van Schil PE, Garg K, Austin JH, Asamura H, Rusch VW, Hirsch FR, Scagliotti G, Mitsudomi T, Huber RM, Ishikawa Y, Jett J, Sanchez-Cespedes M, Sculier JP, Takahashi T, Tsuboi M, Vansteenkiste J, Wistuba I, Yang PC, Aberle D, Brambilla C, Flieder D, *et al*: **International Association for the Study of Lung Cancer/American Thoracic Society/ European Respiratory Society international multidisciplinary classification of lung adenocarcinoma.** *J Thorac Oncol* 2011, **6**:244-285.
2. Yoshizawa A, Motoi N, Riely GJ, Sima CS, Gerald WL, Kris MK, Park BJ, Rusch VW, Travis WD: **Impact of proposed IASLC/ATS/ERS classification of lung adenocarcinoma: prognostic subgroups and implications for further revision of staging based on analysis of 514 stage I cases.** *Mod Pathol* 2011, **24**:653-664.
3. Amin MB, Tamboli P, Merchant SH, Ordóñez NG, Ro J, Ayala AG, Ro JY: **Micropapillary component in lung adenocarcinoma: a distinctive histologic feature with possible prognostic significance.** *Am J Surg Pathol* 2002, **26**:358-364.
4. Miyoshi T, Satoh Y, Okumura S, Nakagawa K, Shirakusa T, Tsuchiya E, Ishikawa Y: **Early-stage lung adenocarcinoma with micropapillary pattern, a distinct pathologic marker for a significantly poor prognosis.** *Am J Surg Pathol* 2003, **27**:101-109.
5. Nagano T, Ishii G, Nagai K, Ito T, Kawase A, Takahashi K, Nishimura Y, Noshiwaki Y, Ochiai A: **Structural and biological properties of a papillary component generating a micropapillary component in lung adenocarcinoma.** *Lung Cancer* 2010, **67**:282-289.
6. Tsutsumida H, Nomoto M, Goto M, Kitajima S, Kubota I, Hirotsu Y, Wakimoto J, Hollingsworth MA, Yonezawa S: **A micropapillary pattern is predictive of a poor prognosis in lung adenocarcinoma, and reduced surfactant apoprotein A expression in the micropapillary pattern is an excellent indicator of a poor prognosis.** *Mod Pathol* 2007, **20**:638-647.
7. Kuba S, Ohtani H, Yamaguchi J, Hayashi H, Uga T, Kanematsu T, Shimokawa I: **Incomplete inside-out growth pattern in invasive breast carcinoma: association with lymph vessel invasion and recurrence-free survival.** *Virchows Arch* 2011, **458**:159-169.
8. Guo X, Chen L, Lang R, Fan Y, Zhang X, Fu : **Invasive micropapillary carcinoma of the breast: association of pathologic features with lymph node metastasis.** *Am J Clin Pathol* 2006, **126**:740-746.
9. Sangoi AR, Beck AH, Amin MB, Cheng L, Epstein JI, Hansel DE, Iczkowski KA, Lopez-Beltran A, Oliva E, Paner GP, Reuter VE, Ro JY, Shah RB, Shen SS, Tamboli P, McKenney JK: **Interobserver reproducibility in the diagnosis of invasive micropapillary carcinoma of the urinary tract among urologic pathologists.** *Am J Surg Pathol* 2010, **34**:1367-1376.
10. Amin MB, Ro JY, el-Sharkawy T, Lee KM, Troncoso P, Silva EG, Ordóñez NG, Ayala AG: **Micropapillary variant of transitional cell carcinoma of the urinary bladder. Histologic pattern resembling ovarian papillary serous carcinoma.** *Am J Surg Pathol* 1994, **18**:1224-1232.
11. Burks RT, Sherman ME, Kurman RJ: **Micropapillary serous carcinoma of the ovary. A distinctive low-grade carcinoma related to serous borderline tumors.** *Am J Surg Pathol* 1996, **20**:1319-1330.
12. Eichhorn JH, Bell DA, Young RH, Scully RE: **Ovarian serous borderline tumors with micropapillary and cribriform patterns: a study of 40 cases and comparison with 44 cases without these patterns.** *Am J Surg Pathol* 1999, **23**:397-409.
13. Yamamoto H, Uryu H, Segawa Y, Tsuneyoshi M: **Aggressive invasive micropapillary salivary duct carcinoma of the parotid gland.** *Pathology Int* 2008, **58**:322-336.
14. Mogi A, Nabeshima K, Hamasaki M, Uesugi N, Tamura K, Iwasaki A, Shirakusa T, Iwasaki H: **Pleural malignant mesothelioma with invasive micropapillary component and its association with pulmonary metastasis.** *Pathology Int* 2009, **59**:874-879.
15. Kondo T: **Bile duct adecarcinoma with minor micropapillary component: a case report.** *Cases Journal* 2009, **2**:51.
16. Shimoda M, Okada Y, Hayashi Y, Hatano S, Kawakubo H, Omori T, Ishii S, Sugiura H: **Primary invasive micropapillary carcinoma of the stomach.** *Pathology Int* 2008, **58**:513-517.
17. Sakamoto K, Watanabe M, Cruz CD, Honda H, Ise H, Mitsui K, Namiki K, Mikami Y, Moriya T, Sasano H: **Primary invasive micropapillary carcinoma of the colon.** *Histopathology* 2005, **47**:479-484.
18. Verdú M, Roman R, Calvo M, Rondon N, Garcia B, Gonzalez M, Vidal A, Puig X: **Clinicopathological and molecular characterization of colorectal micropapillary carcinoma.** *Mod Pathol* 2011, **24**:729-738.
19. Kim MJ, Hong SM, Jang SJ, Yu E, Kim JS, Kim KR, Gong G, Ro JY: **Invasive colorectal micropapillary carcinoma: an aggressive variant of adenocarcinoma.** *Hum Pathol* 2006, **37**:809-815.
20. Sobin LH, Gospodarowicz MK, Wittekind C, editors: **TNM classification of malignant tumors, seventh edition.** Wiley-Blackwell; 2009.

21. Matsukuma S, Yoshihara M, Kasai F, Kato A, Yoshida A, Akaike M, Kobayashi O, Nakayama H, Sakuma Y, Yoshida T, Kameda Y, Ysuchiya E, Miyagi Y: **Rapid and simple detection of hot spot mutations of epidermal growth factor receptor, BRAF, and NRAS in cancers using the loop-hybrid mobility shift assay.** *J Mol Diagn* 2006, **8**:504-512.
22. Goldmann Torsten, Kähler Daniel, Schultz Holger, Abdullah Mahdi, Lang SDagmar, Stellmacher Florian, Vollmer Ekkehard: **On the significance of Surfactant Protein-A within the human lungs.** *Diagnostic Pathology* 2009, 4:8-12.

doi:10.1186/1746-1596-6-92
Cite this article as: Ohe *et al.*: Stromal micropapillary pattern predominant lung adenocarcinoma - a report of two cases. *Diagnostic Pathology* 2011 6:92.

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

Ryo Maeda, MD, Junji Yoshida, MD, PhD,* Genichiro Ishii, MD, PhD,†*
Tomoyuki Hishida, MD, PhD, Mitsuyo Nishimura, MD, PhD,* and Kanji Nagai, MD, PhD**

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.

(*J Thorac Oncol.* 2011;6: 735–742)

*Department of Thoracic Oncology, National Cancer Center Hospital East; and †Department of Pathology, Research Center for Innovative Oncology, National Cancer Center Hospital East, Kashiwa, Chiba, Japan.

Disclosure: The authors declare no conflicts of interest.

Address for correspondence: Junji Yoshida, MD, PhD, Department of Thoracic Oncology, National Cancer Center Hospital East, 6-5-1 Kashiwanoha, Kashiwa, Chiba 277-8577, Japan. E-mail: jyoshida@east.ncc.go.jp

ISSN: 1556-0864/11/0604-0735

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

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

PATIENTS AND METHODS

Patients

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

Pathological Evaluations

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

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

Patient Follow-Up

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

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

Clinicopathological Information

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

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

Statistical Analysis

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

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

RESULTS

Smoking Extent and Clinicopathological Factors

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

TABLE 1. Patient Characteristics and Smoking Extent

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

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

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

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

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

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

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

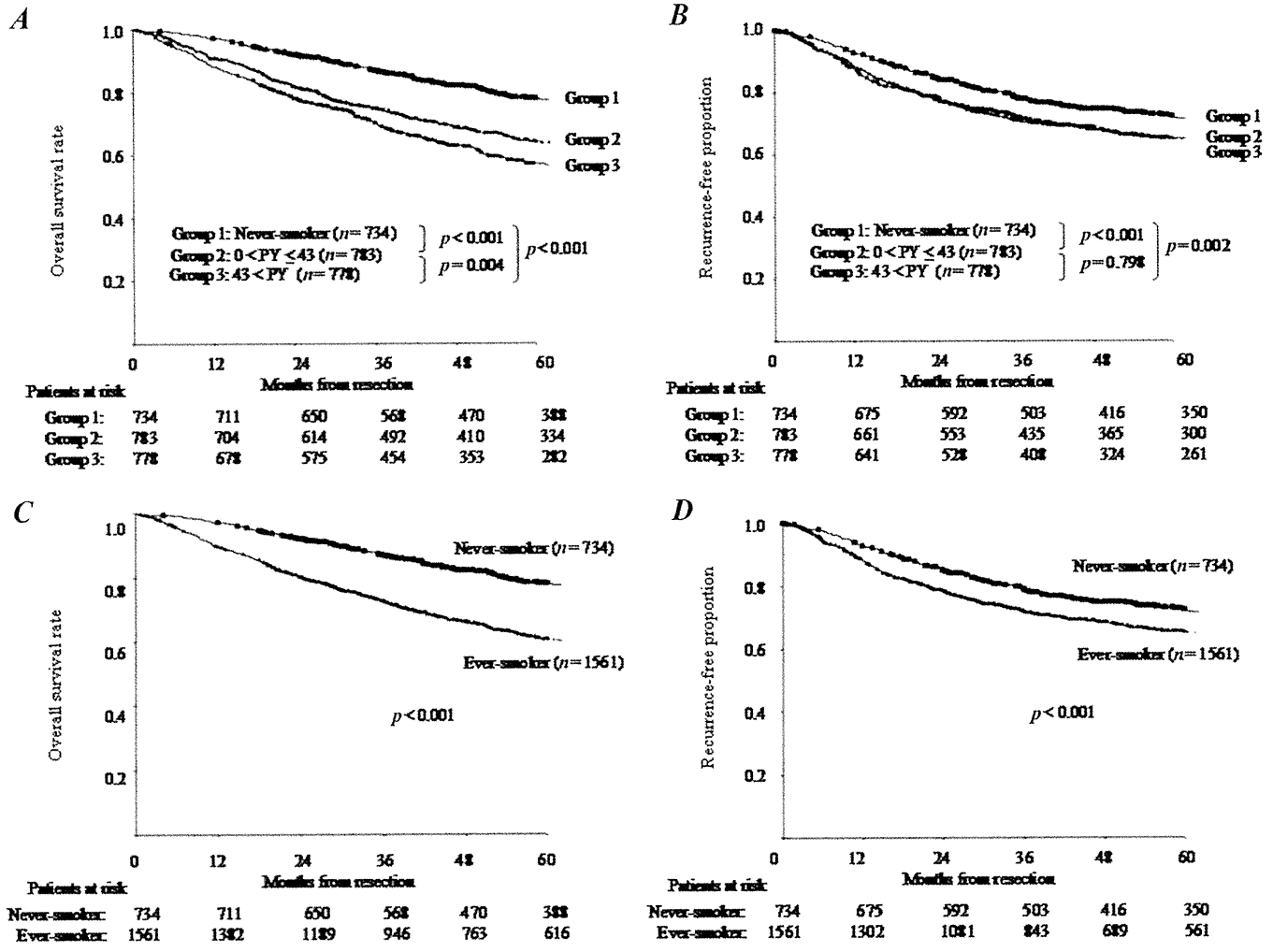

FIGURE 1. Overall survival and recurrence-free proportion (RFP) curves according to smoking status in the entire cohort. *A*, Overall survival curves according to smoking extent. *B*, RFP curves according to smoking extent. *C*, Overall survival curves according to smoking history. *D*, RFP curves according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

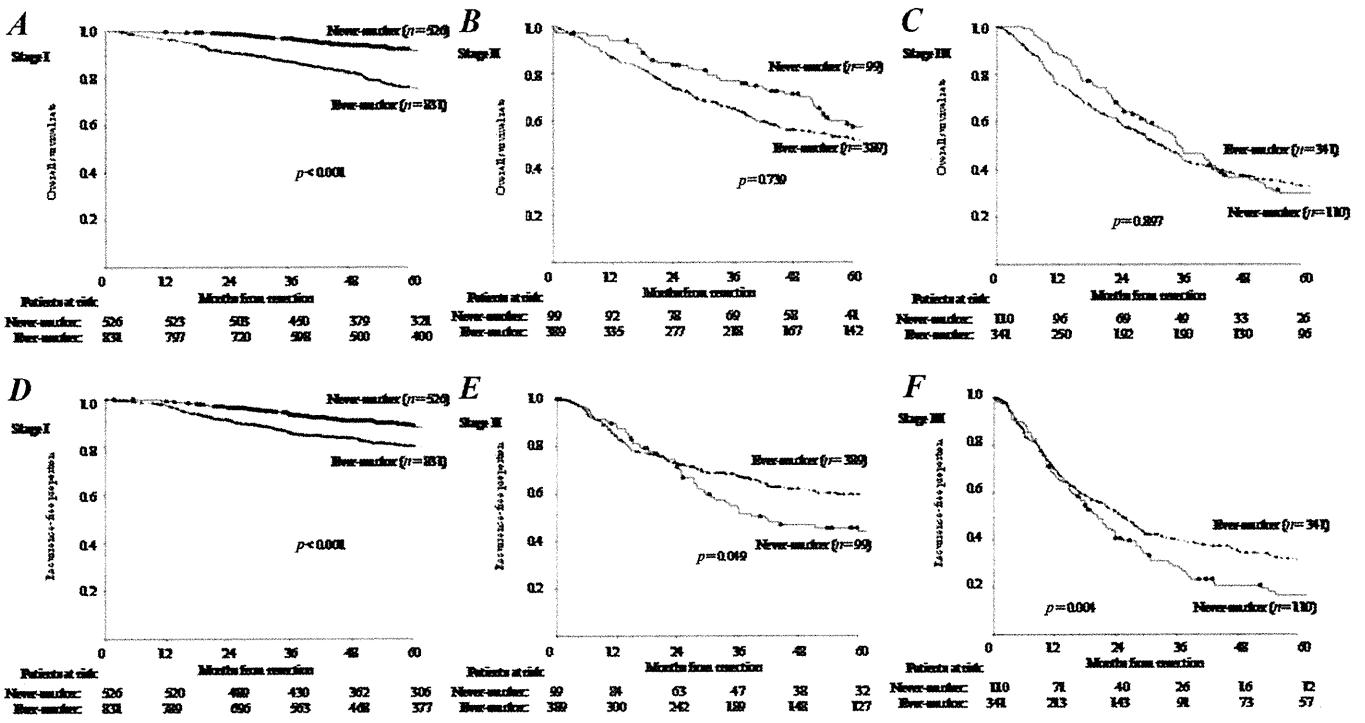

FIGURE 2. Overall survival and recurrence-free proportion (RFP) curves according to smoking history in each stage. *A*, Overall survival curves of patients with stage I non-small cell lung cancer (NSCLC). *B*, Overall survival curves of patients with stage II NSCLC. *C*, Overall survival curves of patients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

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

Prognostic Impact of Cigarette Smoking on Patients with Stage I NSCLC

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

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

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

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

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

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

DISCUSSION

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

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

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

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

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

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

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

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

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

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

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

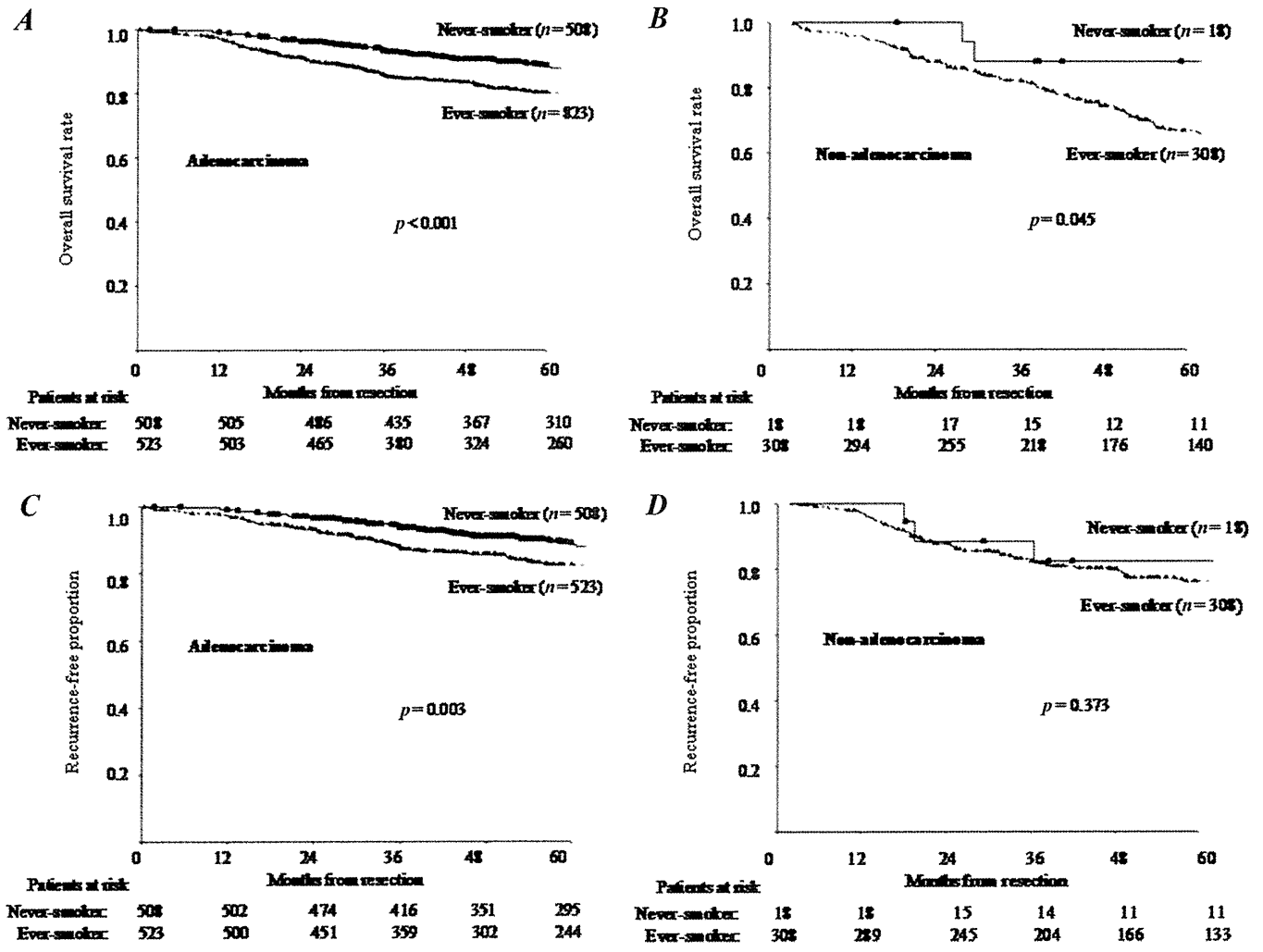

FIGURE 3. Overall survival and recurrence-free proportion (RFP) curves according to smoking history in patients with stage I non-small cell lung cancer. *A*, Overall survival curves of adenocarcinoma patients. *B*, Overall survival curves of nonadenocarcinoma patients. *C*, RFP curves of adenocarcinoma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

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

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

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

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

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

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

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

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

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

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

CONCLUSION

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

ACKNOWLEDGMENTS

Supported, in part, by a Grant-in-Aid for Cancer Research from the Ministry of Health, Labor and Welfare, Japan.

All work included in this article is performed at National Cancer Center Hospital East, Kashiwa, Chiba, Japan. The research was approved by the internal review board of the institution. No patient consent was required as the research is a retrospective chart review and no personally identifiable information was included in the article.

REFERENCES

1. Pfeifer GP, Denissenko MF, Olivier M, et al. Tobacco smoke carcinogens, DNA damage and p53 mutations in smoking-associated cancers. *Oncogene* 2002;21:7435–7451.
2. Stellman SD, Resnicow K. Tobacco smoking, cancer and social class. *IARC Sci Publ* 1997:229–250.
3. Margetts BM, Jackson AA. Interactions between people's diet and their smoking habits: the dietary and nutritional survey of British adults. *BMJ* 1993;307:1381–1384.

4. Ogle KS, Swanson GM, Woods N, et al. Cancer and comorbidity: redefining chronic diseases. *Cancer* 2000;88:653–663.
5. Meliska CJ, Stunkard ME, Gilbert DG, et al. Immune function in cigarette smokers who quit smoking for 31 days. *J Allergy Clin Immunol* 1995;95:901–910.
6. Hanagiri T, Sugio K, Mizukami M, et al. Significance of smoking as a postoperative prognostic factor in patients with non-small cell lung cancer. *J Thorac Oncol* 2008;3:1127–1132.
7. Wu YC, Lin CF, Hsu WH, et al. Long-term results of pathological stage I non-small cell lung cancer: validation of using the number of totally removed lymph nodes as a staging control. *Eur J Cardiothorac Surg* 2003;24:994–1001.
8. Sobue T, Yamamoto S, Hara M, et al.; JPHC Study Group. Japanese Public Health Center. Cigarette smoking and subsequent risk of lung cancer by histologic type in middle-aged Japanese men and women: the JPHC study. *Int J Cancer* 2002;99:245–251.
9. Nordquist LT, Simon GR, Cantor A, et al. Improved survival in never-smokers vs current smokers with primary adenocarcinoma of the lung. *Chest* 2004;126:347–351.
10. Sardari Nia P, Weyler J, Colpaert C, et al. Prognostic value of smoking status in operated non-small cell lung cancer. *Lung Cancer* 2005;47: 351–359.
11. Goldstraw P, Crowley J, Chansky K, et al. The IASLC Lung Cancer Staging Project: proposals for the revision of the TNM stage groupings in the forthcoming (seventh) edition of the TNM Classification of malignant tumours. *J Thorac Oncol* 2007;2:706–714.
12. Travis WD, Brambilla E, Muller-Hermelink HK, et al. World Health Organization Classification of Tumors: Pathology and Genetics of Tumors of the Lung, Pleura, Thymus and Heart. Lyon: IARC Press, 2004.
13. Harpole DH Jr, Herndon JE II, Wolfe WG, et al. A prognostic model of recurrence and death in stage I non-small cell lung cancer utilizing presentation, histopathology, and oncoprotein expression. *Cancer Res* 1995;55:51–56.
14. Tammemagi MC, McLaughlin JR, Mullen JB, et al. A study of smoking, p53 tumor suppressor gene alterations and non-small cell lung cancer. *Ann Epidemiol* 2000;10:176–185.
15. Langendijk JA, Thunnissen FB, Lamers RJ, et al. The prognostic significance of accumulation of p53 protein in stage III non-small cell lung cancer treated by radiotherapy. *Radiother Oncol* 1995;36:218–224.
16. Guo NL, Tosun K, Horn K. Impact and interactions between smoking and traditional prognostic factors in lung cancer progression. *Lung Cancer* 2009;66:386–392.
17. Bryant A, Cerfolio RJ. Differences in epidemiology, histology, and survival between cigarette smokers and never-smokers who develop non-small cell lung cancer. *Chest* 2007;132:185–192.
18. Brundage MD, Davies D, Mackillop WJ. Prognostic factors in non-small cell lung cancer: a decade of progress. *Chest* 2002;122:1037–1057.
19. Westra WH, Slebos RJ, Offerhaus GJ, et al. K-ras oncogene activation in lung adenocarcinomas from former smokers. Evidence that K-ras mutations are an early and irreversible event in the development of adenocarcinoma of the lung. *Cancer* 1993;72:432–438.
20. Suzuki H, Takahashi T, Kuroishi T, et al. p53 mutations in non-small cell lung cancer in Japan: association between mutations and smoking. *Cancer Res* 1992;52:734–736.
21. Tollerud DJ, Clark JW, Brown LM, et al. Association of cigarette smoking with decreased numbers of circulating natural killer cells. *Am Rev Respir Dis* 1989;139:194–198.
22. Lee JS, Yoon A, Kalapurakal SK, et al. Expression of p53 oncoprotein in non-small-cell lung cancer: a favorable prognostic factor. *J Clin Oncol* 1995;13:1893–1903.
23. Pezzella F, Turley H, Kuzu I, et al. bcl-2 protein in non-small-cell lung carcinoma. *N Engl J Med* 1993;329:690–694.
24. Hsu HS, Wen CK, Tang YA, et al. Promoter hypermethylation is the predominant mechanism in hMLH1 and hMSH2 deregulation and is a poor prognostic factor in nonsmoking lung cancer. *Clin Cancer Res* 2005;11:5410–5416.
25. Sato M, Shames DS, Gazdar AF, et al. A translational view of the molecular pathogenesis of lung cancer. *J Thorac Oncol* 2007;2:327–343.
26. Wang CY, Huang MS, Huang MH, et al. Persistently high serum carcinoembryonic antigen levels after surgery indicate poor prognosis in patients with stage I non-small-cell lung cancer. *J Surg Res* 2010;163: e45–e50.
27. Dhital K, Saunders CA, Seed PT, et al. [(18)F]Fluorodeoxyglucose positron emission tomography and its prognostic value in lung cancer. *Eur J Cardiothorac Surg* 2000;18:425–428.
28. Al-Sarraf N, Gately K, Lucey J, et al. Clinical implication and prognostic significance of standardized uptake value of primary non-small cell lung cancer on positron emission tomography: analysis of 176 cases. *Eur J Cardiothorac Surg* 2008;34:892–897.
29. Higashiyama M, Kodama K, Yokouchi H, et al. Prognostic value of bronchiolo-alveolar carcinoma component of small lung adenocarcinoma. *Ann Thorac Surg* 1999;68:2069–2073.
30. Sakao Y, Miyamoto H, Sakuraba M, et al. Prognostic significance of a histologic subtype in small adenocarcinoma of the lung: the impact of nonbronchioloalveolar carcinoma components. *Ann Thorac Surg* 2007; 83:209–214.
31. Anami Y, Iijima T, Suzuki K, et al. Bronchioloalveolar carcinoma (lepidic growth) component is a more useful prognostic factor than lymph node metastasis. *J Thorac Oncol* 2009;4:951–958.

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

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

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

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

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

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

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

(*J Thorac Oncol.* 2011;6: 743–750)

*Department of Thoracic Oncology, National Cancer Center Hospital East; and †Department of Pathology, Research Center for Innovative Oncology, National Cancer Center Hospital East, Kashiwa, Chiba, Japan.

Disclosure: The authors declare no conflicts of interest.

Address for correspondence: Genichiro Ishii, MD, PhD, Department of Pathology, Research Center for Innovative Oncology, National Cancer Center Hospital East, Kashiwa, Chiba, Japan. E-mail: gishii@east.ncc.go.jp

ISSN: 1556-0864/11/0604-0743

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

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

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

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

PATIENTS AND METHODS

Patients Selection

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

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

Pathological Evaluations

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

Clinicopathological Information

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

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

Statistical Analysis

Differences in categorical outcomes were evaluated by χ^2 test. Continuous variables were compared using t tests. To offset the prognostic impact of comorbidities associated with cigarette smoking, we investigated recurrence-free probabilities for this study. The length of recurrence-free probability was calculated in months from the date of resection to the date of first recurrence or last follow-up. To calculate the recurrence-free probability, patients who died without recurrence or who were known to be recurrence free at the date of last contact were excluded from the calculation. For univariate analyses, all recurrence-free probabilities were estimated using the Kaplan–Meier method, and comparisons of these variables were made using the log-rank test. Multivariate analyses were performed using Cox's proportional hazard regression model. Clinical predictors for the presence of a solid component were evaluated by logistic regression analyses. The predictors from univariate analyses were also evaluated using multiple regression analyses. The p value less than 0.2 in a univariate model was set as the threshold used for selection of variables in a multivariate model. All reported p values were two sided, and the significance level was set at less than 0.05. Analyses were performed using SPSS version 11.0 (Dr. SPSS II for Windows, standard version 11.0, SPSS Inc., Chicago, IL) and GraphPad Prism (Prism for Windows, Version 5.02, GraphPad Software, Inc., La Jolla, CA).

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

RESULTS

Patient Characteristics and Recurrence-Free Probabilities According to Clinicopathological Factors

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

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