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ABTS Requirements for the 10-Year Milestone for Maintenance of Certification

Diplomates of the American Board of Thoracic Surgery (ABTS) who plan to participate in the 10-Year Milestone for the Maintenance of Certification (MOC) process as Certified-Active must hold an unrestricted medical license in the locale of their practice and privileges in a hospital accredited by the JCAHO (or other organization recognized by the ABTS). In addition, a valid ABTS certificate is an absolute requirement for entrance into the MOC process. If your certificate has expired, the only pathway for renewal of a certificate is to take and pass the Part I (written) and the Part II (oral) certifying examinations.

The CME requirements are 150 Category I credits over a five-year period. At least half of these CME hours need to be in the broad area of thoracic surgery. Category II credits are not accepted. Interested individuals should refer to the Board's website (www.abts.org) for a complete description of acceptable CME credits.

Diplomates will be required to take and pass a secured exam after their application has been approved. Taking SESATS in lieu of the secured exam is not an option. The secured exam is administered over a two-week period in September of every year at Pearson Vue Testing Centers, which are located nationwide. Diplomates will have the opportunity to select the day and location of their exam. For the dates of the next MOC exam, visit the Board's web site at www.abts.org.

Starting on July 1, 2014, the ABTS will require its Diplomates to participate in an outcomes database as fulfillment of Part IV (Performance in Practice) for the 10-year Milestone of Maintenance of Certification (MOC). For a list of approved outcomes databases or for more information on how to have a database approved by the Board, visit the Board's website at www.abts.org. Participation in the Professional Portfolio will no longer be accepted as fulfillment of MOC Part IV after July 1, 2014.

Diplomates may apply for MOC in the year their certificate expires or, if they wish to do so, they may apply up to two years before it expires. However, the new certificate will be dated 10 years from the date of expiration of their original certificate or most recent MOC certificate. In other words, going through the MOC process early does not alter the 10-year validation. Diplomates certified prior to 1976 (the year that time-limited certificates were initiated) are also required to participate in MOC if they wish to maintain valid certificates.

The deadline for submitting an application for 10-year Milestone of MOC is March 15 of every year. Information outlining the rules, requirements, and dates for MOC in thoracic surgery is available on the Board's website at www.abts.org. For additional information, please contact the American Board of Thoracic Surgery, 633 N St. Clair St, Ste 2320, Chicago, IL 60611; telephone (312) 202-5900; fax (312) 202-5960; e-mail: info@abts.org.

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ORIGINAL ARTICLE

Significance of tumour vessel invasion in determining the morphology of isolated tumour cells in the pulmonary vein in non-small-cell lung cancer

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Abstract

OBJECTIVES: The existence of clustered isolated tumour cells (ITCs) in the pulmonary vein (PV) of the lungs of patients with lung cancer has been reported to be a prognostic factor. However, the clinical–pathological characteristics related to their presence in the PV remain unclear.

METHODS: We analysed the surgical results and clinical–pathological findings of 130 patients who underwent surgery for non-small-cell lung cancer in regard to blood vessel invasion (BVI), serum carcinoembryonic antigen (CEA) level, maximum standardized uptake value (SUV-max), size of the solid region in computed tomography findings and pathological stage according to an ITC type, i.e. no tumour (N), singular tumour cells (S) and clustered tumour cells (C).

RESULTS: ITCs were detected in 96 (74%) of the patients, with C observed in 43, S in 53 and N in 34. Recurrence was seen in 33 (26%) cases, 21 of which were classified as C, 9 as S and 3 as N. The disease-free survival rate was significantly worse in C cases when compared with the others ($P < 0.01$). The rate of C was high in cases with high serum CEA, advanced p-staging and positive BVI ratio. Furthermore, BVI positive and ITC morphology were strongly related (BVI positive; 79 in C, 40 in S, 9% in N; $P < 0.01$).

CONCLUSIONS: Clustered ITCs were shown to be a prognostic indicator and strongly related to BVI. Our results suggest that determination of BVI has prognostic value, as clustered ITCs with metastatic potential are disseminated from the invaded vein.

Keywords: Blood vessel invasion • Isolated tumour cells • Surgery • Recurrence • Non-small-cell lung cancer

INTRODUCTION

Lung cancer is a leading cause of cancer death in most industrial countries [1]. In addition, an investigation of the causes of cancer deaths indicated that recurrence and distant metastasis occurred in approximately 70% of patients who underwent surgery [2]. Therefore, useful markers are needed for the early detection of distant metastasis and recurrence, with various prognostic biomarkers thus far reported. In blood chemical studies, serum carcinoembryonic antigen (CEA) has been shown to be one of the most useful tumour markers for providing information regarding cancer progression [3], while the presence of blood vessel invasion (BVI) in histopathological findings is also an important prognostic indicator [4, 5]. On the other hand, clinical imaging techniques such as computed tomography (CT) and positron emission tomography (PET) can also provide important information regarding cancer aggressiveness and malignancy. The solid lesion size of tumours in CT findings and maximum standardized uptake value (SUV-max) in PET imaging are helpful for clinical cancer treatment, as those factors are reported to reflect malignancy or metastatic potential [6, 7]. As for other biomarkers, the presence of isolated tumour cells (ITCs) in blood has been recently reported to be useful for determining prognosis, recurrence and metastasis [8–11]. In our previous report, we presented a novel method to enrich ITCs while maintaining their morphological appearance and then found relationships between ITC morphology and clinical backgrounds [12]. In cases with clustered ITCs, the recurrence rate was higher than that in cases with singular or no ITCs. From those results, we speculated that ITCs are shed from the primary tumour, then flow through a drainage vein and circulate throughout the whole body, easily leading to metastasis. In addition, relationships between ITCs and background factors of primary tumours were noted. In the present study, we assessed the relationships among clinical–pathological findings including BVI and morphological characteristics of ITCs in the pulmonary vein (PV) of resected lungs of non-small-cell lung cancer patients.

Table 1: Patient characteristics and distribution of ITCs in PV blood

	Total	PV cytology			*P*-value
		N	S	C	
Number	130	34	53	43	
Gender					
Male	74	22	26	26	0.8
Female	56	12	27	17	
Age in years (mean ± SD)	68 ± 9.0	67 ± 9.2	67 ± 9.3	69 ± 11.0	0.3
P-stage					
I	98	30	42	26	0.01
II	20	3	5	12	
III or IV	12	1	6	5	
Tumour histology					
Adenocarcinoma	92	21	41	30	0.8
Squamous cell carcinoma	26	8	10	8	
Miscellaneous	12	5	2	5	

PV: pulmonary vein; N: no tumour cells; S: singular tumour cells; C: clustered tumour cells.

MATERIALS AND METHODS

Detection of ITCs

The methods used for the detection of ITCs were reported in detail in our previous study [12]. Briefly, 50 µl/ml of a RosetteSep® Human CD45 Depletion Cocktail (Stemcell Technologies, Inc., Vancouver, Canada) was added to individual whole blood samples and mixed well. After incubation at room temperature, the mixture was diluted with an equal volume of phosphate buffered saline plus 2% fetal bovine serum and mixed gently. The diluted sample was then layered on the top of a Ficoll-Paque™ PLUS and centrifuged, then enriched ITCs were removed from the Ficoll-Paque™ PLUS–plasma interface. The cells were centrifuged down to polylysine-coated glass slides using a cytospin device. Cells on the slides were subjected to Papanicolaou staining.

Patients

One hundred and thirty consecutive patients (56 males, 74 females; range 28–88 years old, median 68.0 years) with primary non-small-cell lung cancer who did not undergo preoperative chemotherapy and/or radiation therapy were evaluated using our method (Table 1). Written informed consent was obtained from all enrolled patients. This study conformed to the ethical guidelines of Osaka University Graduate School of Medicine and was approved by the institutional review board of Osaka University Medical Hospital. All patients underwent a segmentectomy ($n = 11$), lobectomy or bilobectomy ($n = 119$) during systematic mediastinal lymphadenectomy procedures performed from August 2008 to 2010 at Osaka University Medical Hospital.

Postoperative staging of all patients was determined according to the tumour-node-metastasis (TNM) classification of the Union for International Cancer Control, ver. 7, 2009 (Table 1). The median follow-up duration was 19 (6–22 months). In follow-up examinations, all patients were evaluated at 3-month intervals. Each evaluation included a physical examination, chest X-ray and blood tests including tumour markers, while additional thoraco-abdominal CT scans were generally performed at 6-month intervals.

Blood samples, and ITC detection and enrichment

All blood samples were collected immediately after tumour resection by gently aspirating from the tumour-draining PV using an 18-gauge needle and placed in 10-ml ethylenediaminetetraacetic acid tubes. ITCs were isolated using a negative selection method from 1-ml blood samples using the method described in Section 2.1.

Evaluation and classification of clusters

Using all of the samples, one glass slide containing enriched ITCs from each patient was prepared and assessed by Papanicolaou staining. These examinations were performed independently by 2 cytologists (Eiichi Morii and Hideo Yoshimura) who were unaware of the patient's clinical data. For morphological assessment, each cytologist distinguished cancer cells from normal cells by light microscopy based on their morphological appearance, such as cell size and shape, nuclear size and shape and nuclear-cytoplasmic ratio. Furthermore, for cluster formation assessment, patterns of ITCs were classified into the following three types: no tumour cells (N), singular tumour cells (S) and clustered tumour cells (C). The correlations of patient characteristics with the distribution of ITC morphology in pulmonary venous blood are shown in Table 1. There were no significant differences regarding patient characteristics excluding p-staging among the three groups (N, S and C).

Clinical–pathological analyses according to morphological appearance

To reveal the relationships between clinical–pathological findings and ITC morphology, we examined the following parameters: serum CEA, size of solid primary tumour in CT findings, pathological stage and BVI evaluated by haematoxylin and eosin or elastic van Gieson staining. In patients with CEA ≥5 ng/ml, solid lesion size ≥20 mm in diameter and SUV-max ≥2.5 were considered to be elevated values [7, 13]. Statistical analysis was

performed using commercially available statistics software (JMP version 9, SAS Institute). A *P*-value <0.05 was considered to indicate a statistically significant difference. The characteristics of the patients were compared using the *t*-test, chi-square test and Fisher's exact test. Tukey–Kramer was used to compare the mean values of groups. For the analysis of follow-up data, survival curves were calculated using the Kaplan–Meier method, and survival distributions were compared with a log-rank test. In addition to examining the relationships among ITC morphology and clinical-pathological findings, we utilized logistic regression analysis. Furthermore, to examine the risk ratios of recurrence, a multivariate Cox's hazard model was used.

RESULTS

ITC classification and prognosis

ITCs classified as S were detected in 53 of the 130 patients (40%), while those classified as C were found in 43 (33%). During the median follow-up period of 19 months, 33 patients suffered cancer relapse, 21 in the C group, 9 in S and 3 in N. In the relapsed cases, exclusive local recurrence occurred in 11 (pleura in 7, chest wall in 2, hilar lymph node in 2, mediastinal lymph node in 1), local and distant metastases in 8 (bilateral lungs in 3, mediastinal lymph node and neck lymph node in 4, mediastinal lymph node and bone in 1) and exclusive distant metastasis in 13 (contralateral lung in 4, adrenal grand in 3, brain and liver in 2, other organs in 4). Relapse-free survival curves demonstrated that survival in the C group was significantly worse (Fig. 1). Table 2 also shows the 2-year recurrence free rate according to clinical parameters obtained during the follow-up period. From those results, all of the examined parameters showed an adequate predictive value.

Relationships of ITC morphology with clinical and histopathological findings

The relationships among ITC morphology, clinical background and histopathological findings are shown in Fig. 2. There were significant differences regarding ITC morphology for the clinical parameters serum CEA, positive BVI ratio and advanced p-stage. In cases classified as C, the levels of those parameters were elevated when compared with the N and S cases. In contrast, there were no significant differences regarding ITC morphology for elevated SUV-max and solid lesion size (Fig. 2). In addition, multivariate analysis revealed BVI to be one of the most important factors to determine the ITC morphology (Table 3).

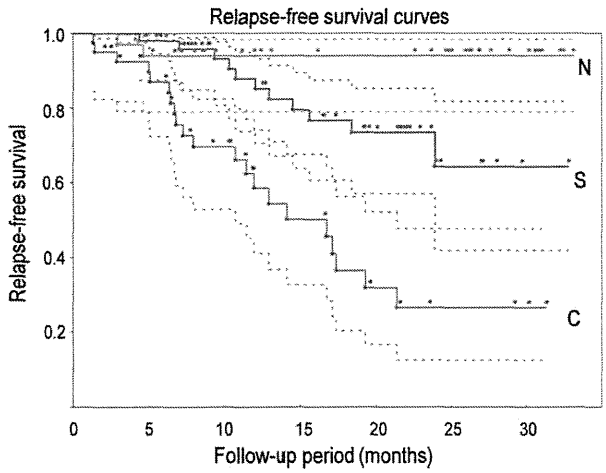

Figure 1: Relapse-free survival curves according to ITC morphology. N: no tumour cells; S: singular tumour cells; C: clustered tumour cells.

DISCUSSION

The present findings of blood samples taken from the PV of resected lungs of patients with lung cancer showed that the morphological pattern of ITCs was correlated with the clinical-pathological parameters for BVI. These results may provide new and important information regarding ITCs in the blood of affected patients.

The presence of ITCs in blood has been reported to be a useful biomarker in lung cancer patients [8–11]. We previously reported that ITCs in the PV were detected using a CD45-negative selection method and found that not only their presence but also cluster formation may be a prognostic indicator for early recurrence of lung cancer [12]. In cases with singular cancer cells, the recurrence rate was low when compared with those with cluster formation. However, it remains unclear whether ITC morphology is related to histopathological findings or clinical background. In the present study, we mainly investigated the relationships between the ITC morphological appearance and clinical pathological findings.

Recent studies have found that ITCs in blood have morphological and biological diversity. Hou *et al.* [14] used a filtration method (isolation by size of epithelial tumor cells [ISET]) to detect and enrich circulating tumour cells (CTCs) according to

Table 2: Relapse-free survival according to clinical-pathological parameters

	Variables	*N*	2Y-RFS (%)	*P*-value
1	ITCs in PV			<0.001
	None (N)	34	93	
	Singular (S)	53	64	
	Clustered (C)	44	26	
2	P-stage			<0.001
	Early (I)	98	71	
	Advanced (II, III, IV)	32	35	
3	Serum CEA			0.03
	Low (<5 ng/ml)	103	66	
	High (≥5 ng/ml)	25	43	
4	SUV-max			<0.001
	Low (<5)	51	77	
	High (≥5)	43	31	
5	BVI			<0.001
	Negative	71	92	
	Positive	59	30	
6	Solid lesion			<0.001
	<20 mm	81	87	
	≥20 mm	49	46	

2Y-RFS: 2-year relapse-free survival; ITCs: isolated tumour cells; PV: pulmonary vein; SUV: standardized uptake value; BVI: blood vessel invasion; Solid lesion: size of solid lesion in CT findings.

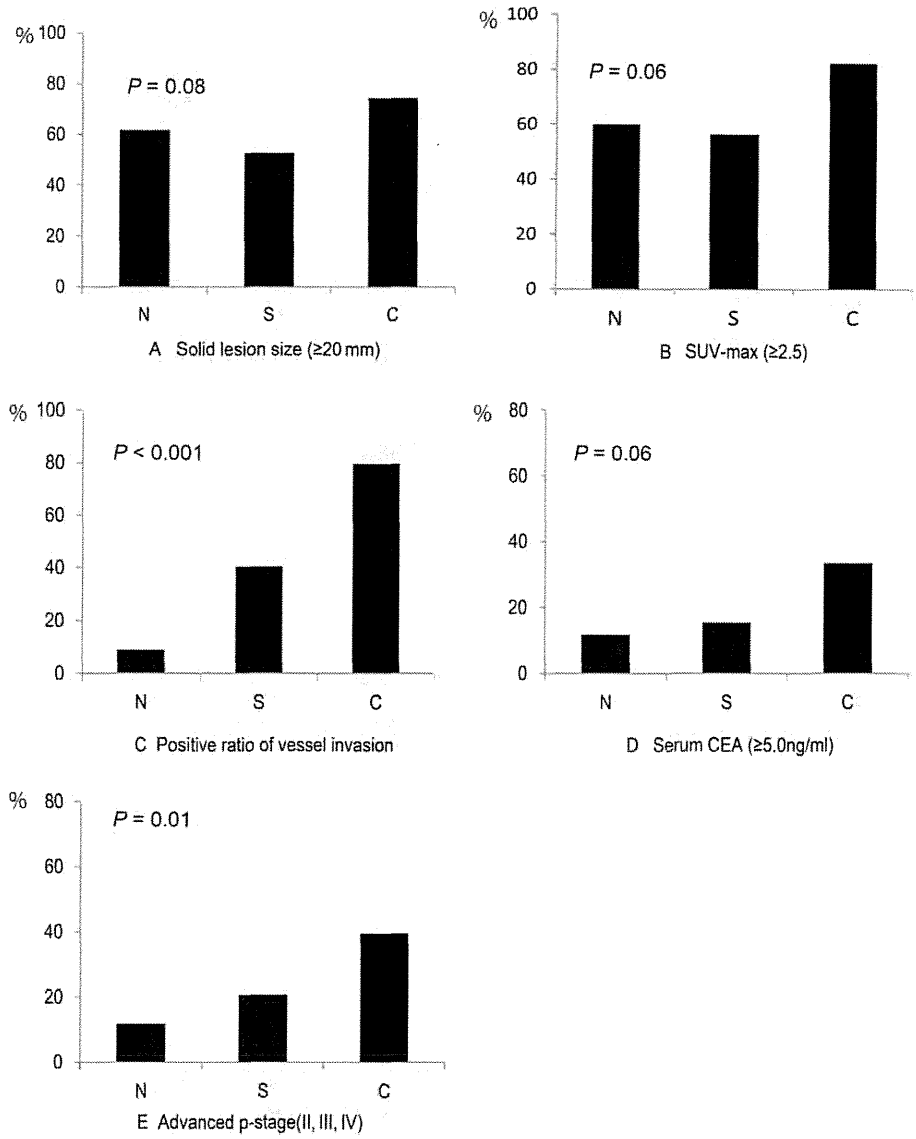

Figure 2: Relationships among ITC morphology and clinical parameters. The vertical axis indicates a high or positive ratio of each individual parameter. (**A**) Solid lesion size in CT findings (≥20 mm). (**B**). SUV-max in PET findings (≥5). (**C**) Blood vessel invasion. (**D**) Serum CEA (≥5 ng/ml). (**E**) Advanced pathological stage (II/III/IV).

the cell size obtained from the blood of lung cancer patients and then analysed their biological characteristics. Based on immunochemical staining results, they showed that clustered CTCs survive longer and have greater anti-apoptosis potential when compared with singular cells. Their findings correspond to our previous results.

As for other predictive factors, histopathological analyses have also provided important information about the biological characteristics of primary cancer. Table 2 shows the factors indicated to have a significant predictive value in the present study, with BVI found to be one of the most revealing prognostic biomarkers. Several groups have reported that BVI was a useful predictive factor for recurrence and metastasis [4, 5]. However, the related mechanisms have not been fully elucidated. In the present study, we found that the presence of clustered cancer cells was strongly related to BVI by primary tumours, as the positive ratio of BVI was significantly higher in cases with clustered ITCs when compared with the N and S groups. Our results showed that clustered ITCs in blood were seen in most cases with BVI, whereas singular cancer cells were also found in cases without BVI. This discrepancy may be because singular ITCs are more easily shed from microvascular vessels around the primary tumour that are too small to be diagnosed as positive BVI, while clustered ITCs may enter the bloodstream mainly as a result of large vessel invasion in an amount adequate for a BVI positive diagnosis. Our findings suggest that BVI indicates not only the presence of singular cancer cells in the bloodstream, but also clustered cancer cells with metastasis potential. In addition, clustered cancer cells may be easily shed from the invaded vein into the bloodstream in cases with vessel invasion, resulting in metastasis. For further analysis of the prognostic recurrent value of these clinical parameters, risk ratios after excluding apparent local recurrence cases were calculated using the Cox's proportional hazard model (Table 4). Those results also revealed BVI as

Table 3: Multivariate analysis of clinical-pathological parameters related to morphology of ITCs

	P-value	Odds ratio	95% CI
S/N			
BVI (+)	<0.01	5.85	1.54–30.04
CT (solid >2 cm)	0.25	0.49	0.13–1.65
CEA (>5 ng/ml)	0.31	1.56	0.33–8.68
P-stage (II, III, IV)	0.8	1.19	0.24–6.07
SUV-max (>2.5)	0.8	1.19	0.31–4.75
C/N			
BVI(+)	<0.01	103.38	15.3–2215
CT (solid >2 cm)	0.77	0.73	0.07–5.21
CEA (5 ng/ml)	0.39	2.33	0.34–19.75
P-stage (II, III, IV)	0.56	1.91	0.18–20.3
SUV-max (>2.5)	0.21	0.21	0.008–2.23

CI: confidence interval; N: no tumour cells; S: singular tumour cells; C: clustered tumour cells; BVI: blood vessel invasion; SUV: standardized uptake value.

Table 4: Multivariate analysis of the prognostic value in recurrent cases excluding local recurrence

	P-value	Risk ratio	95% CI
CEA (>5 ng/ml)	0.79	1.16	0.35–3.29
CT (solid >2 cm)	0.31	2.8	0.42–55.54
BVI	0.02	4.27	1.14–20.38
P-stage (II, III, IV)	0.04	2.98	1.01–8.93
SUV-max (>2.5)	0.8	3.54	0.88–24.71

CI: confidence interval; BVI: blood vessel invasion; SUV: standardized uptake value.

an independent predictive factor, while they suggest that the presence of BVI is an important factor to determine the morphology of ITCs in blood and the relationship to distant metastasis.

In conclusion, the presence of ITCs in the PV of resected lungs of lung cancer patients was shown to be related to varied morphologies, while significant relationships were found among clinical factors and histopathological findings. Importantly, BVI may indicate the presence of clustered ITCs. Our results support the notion that BVI is a predictive factor for early recurrence and distant metastasis, as a large number of clustered ITCs with metastatic potential may be disseminated from invaded blood vessels. These findings should be helpful to further elucidate the mechanism of metastasis in lung cancer.

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Conflict of interest: none declared.

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Radiographically determined noninvasive adenocarcinoma of the lung: Survival outcomes of Japan Clinical Oncology Group 0201

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Objective: The study objective was to evaluate the long-term survival of patients with radiographically determined noninvasive lung adenocarcinomas.

Methods: A prospective, multi-institutional study on image diagnosis to define early (noninvasive) adenocarcinomas of the lung (Japan Clinical Oncology Group 0201) has shown that a consolidation/tumor ratio on thin-section computed tomography 0.25 or less in cT1a (≤2.0 cm) could be used as a better radiologic criterion for a noninvasive pathology than a consolidation/tumor ratio 0.50 or less in cT1a-b (≤3.0 cm). From the prognostic viewpoints, these criteria were evaluated for 545 patients with adenocarcinoma who underwent lobectomy and lymph node dissection.

Results: The subjects consisted of 233 men and 312 women with a median age of 62 years. The median follow-up period among overall patients was 7.1 years (range, 0-8.5 years). The overall and relapse-free 5-year survivals of the overall patients were 90.6% and 84.7%, respectively. When a consolidation/tumor ratio 0.5 or less in cT1a-b was used as a cutoff, the 5-year overall survivals of radiologic noninvasive (121 patients, 22.2%) and invasive (424 patients, 77.8%) adenocarcinomas were 96.7% and 88.9%, respectively, and the difference was statistically significant ($P < .001$, log-rank test). With the use of a consolidation/tumor ratio 0.25 or less in cT1a, the 5-year overall survivals of radiologic noninvasive (35 patients, 12.1%) and invasive (254 patients, 87.9%) adenocarcinomas were 97.1% and 92.4%, respectively, and the difference was not statistically significant ($P = .259$).

Conclusions: The radiologic criteria of a consolidation/tumor ratio 0.25 or less in cT1a (≤2.0 cm) and 0.50 in cT1a-b (≤3.0 cm) were both able to define a homogeneous group of patients with an excellent prognosis before surgery. (J Thorac Cardiovasc Surg 2013;146:24-30)

Our understanding of the natural history of small, peripheral adenocarcinomas has greatly progressed.[1-5] This understanding is reflected in the recently revised international multidisciplinary classification of lung adenocarcinoma sponsored by the International Association for the Study of Lung Cancer, American Thoracic Society, and European Respiratory Society.[6] This new classification is characterized by the creation/abandonment of some terminology for early and advanced adenocarcinomas and by a multidisciplinary approach for the application of the new classification in a clinical setting. In particular, the term "bronchioloalveolar carcinoma" is no longer used, and new concepts have been introduced, such as adenocarcinoma in situ and minimally invasive adenocarcinoma. Invasive adenocarcinomas are classified according to the predominant pattern after comprehensive histologic subtyping with lepidic, acinar, papillary, micropapillary, and solid patterns. The term "mixed subtype adenocarcinoma" is no longer used.

In this new proposal, the surgical features have been described as part of a multidisciplinary approach to the comprehensive classification of adenocarcinomas. Especially for early adenocarcinomas, crucial questions have been raised, such as "Is sublobar (limited) resection adequate oncological treatment for some early adenocarcinomas?" and "Can computed tomography (CT) be used to select patients for sublobar resection?"[6,7] The Japan Clinical Oncology Group (JCOG) conducted a prospective, radiologic study of thin-section computed tomography (TSCT) to identify radiologic criteria that predict pathologic noninvasiveness

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Abbreviations and Acronyms
CI = confidence interval
CT = computed tomography
C/T = consolidation/tumor
GGO = ground-glass opacity
JCOG = Japan Clinical Oncology Group
TSCT = thin-section computed tomography

in clinical IA lung cancer arising in the periphery of the lung (JCOG 0201).[8] The current study demonstrates the prognosis of the patients who are selected for limited sublobar resection according to the radiologic criteria for noninvasive adenocarcinomas. If we believe that patients with noninvasive adenocarcinomas may be appropriately treated with limited sublobar surgical resection instead of lobectomy, it is crucially important to answer the question, "Can CT be used to select candidates for sublobar resection or those who can benefit from limited sublobar resection?"

In selecting candidates for sublobar resection for early lung adenocarcinomas, however, the preoperative, radiologic prediction of the degree of invasive growth of the tumor has been realized to be a crucial point. Because of the advent of high-resolution CT or TSCT, the radiologic appearance of adenocarcinomas at various stages has been described in detail, and the radiology-pathology correlation has been studied. A localized, nodular lesion characterized by a low-to-moderate increase in CT density that does not obscure lung structures, such as the pulmonary artery/vein and bronchus, is referred to as "ground-glass opacity" (GGO).[3] It has been shown that GGOs are more likely to be an early form of adenocarcinomas, such as bronchioloalveolar carcinoma, adenocarcinoma in situ, or minimally invasive adenocarcinoma. In particular, some GGOs are accompanied by a solid part, and it has been demonstrated that a solid component represents the portion of invasive growth. These observations suggested that the degree of pathologic invasive growth in adenocarcinoma could be quantified according to the proportion of increased solid density in the CT appearance of the lesion.[9]

On the basis of these observations, the JCOG 0201 study was planned to establish radiologic criteria that could be used to identify pathologic early (noninvasive) adenocarcinomas according to the quantification of the solid component in TSCT. For this purpose, a consolidation/tumor (C/T) ratio on TSCT was used, and the hypothesis that a C/T ratio 0.50 or less in cT1a-b ($\leq$3.0 cm) in the TNM staging system (7th) indicates a noninvasive pathology was tested with specificity as the primary end point. The pathology-radiology correlation has been described.[8] The present study addresses the evaluation of a radiologic criterion in terms of the prognosis after surgery. If we can combine prognostic and pathologic features, we may be able to improve the radiologic criteria for defining noninvasive adenocarcinoma.

PATIENTS AND METHODS

The Japan Clinical Oncology Group 0201 Study

The study design and primary results of the prospective, multiinstitutional study entitled JCOG 0201 have been published.[8] The study protocol was approved by the JCOG Clinical Trial Review Committee and the institutional review board of each participating center. The study was originally intended to define the radiologic criteria that indicate the pathologic noninvasiveness of adenocarcinomas arising in the periphery of the lung. If the radiologic selection of early adenocarcinomas with an excellent prognosis could be achieved, candidates for limited resection, not for lobectomy, could be precisely identified. Therefore, the radiologic definition of noninvasive adenocarcinoma needs to be assessed not only according to the radiology-pathology correlation but also according to the prognosis. The current study describes the prognosis of patients with resected adenocarcinoma according to different radiologic criteria. In brief, patients who met the following criteria were enrolled in the JCOG 0201 study: (1) a suspected or diagnosed lung cancer based on the findings from a plain x-ray or CT scan; (2) clinical stage IA (ie, T1N0M0) by thoracic enhanced CT; (3) the center of the tumor located peripherally (ie, in the outer half of the lung field) on CT; (4) measurable in at least 1 dimension in TSCT; (5) age 20 to 75 years; (6) no prior thoracotomy; (7) feasible for pulmonary lobectomy; and (8) obtained written informed consent. Before surgery, all patients underwent a contrast-enhanced, thin-section helical CT scan with 1- to 3-mm collimation, with particular focus on the primary tumor to estimate the size of the entire tumor, including GGO and the solid part (consolidation), where the C/T ratio was defined as a predictor of pathologic early adenocarcinoma (Figure 1). The patients then underwent surgical resection with at least lobectomy and hilar/mediastinal lymph node dissection. In the JCOG 0201 study, the appropriateness of C/T ratios of 0.50 for cT1a-b ($\leq$3.0 cm) and 0.25 for cT1a ($\leq$2.0 cm) was studied by comparing the C/T ratio and pathology of the resected specimen. The primary end point of the study was the specificity, which was defined as the proportion of patients with radiologically diagnosed invasive lung cancer determined by the central radiologic review among patients with pathologically diagnosed invasive lung cancer. Pathologic noninvasiveness was defined as pN0 disease with neither vascular invasion nor lymphatic permeation on resected specimen.

Patients

Between December 2002 and May 2004, we enrolled 811 patients from 31 institutions. There were 357 men and 454 women (age range, 27-75 years; median, 61 years). Of these, 562 patients (69.3%) underwent major lung resection. After 17 patients who were ineligible on the basis of postoperative pathologic findings were excluded, the prognosis of 545 patients (67.2%) was finally studied according to radiologic criteria for noninvasive adenocarcinoma mainly represented by the C/T ratio. The subjects of this study are the same as those in the analysis of pathology-radiology correlation of JCOG0201.

Statistical Analysis

The method of sample size calculation has been described.[8] Overall survival was defined as the duration from enrollment to death from any cause. For the patients alive, overall survival was censored at the last visit. Relapse-free survival was defined as the duration from enrollment to first recurrence or death from any cause. For the patients alive and recurrence-free, relapse-free survival was censored at the last visit. The probability of survival was estimated by the Kaplan–Meier method, and survival curves were drawn. The difference in survival between the groups was tested by the log-rank test. All P values were 2-sided. All statistical

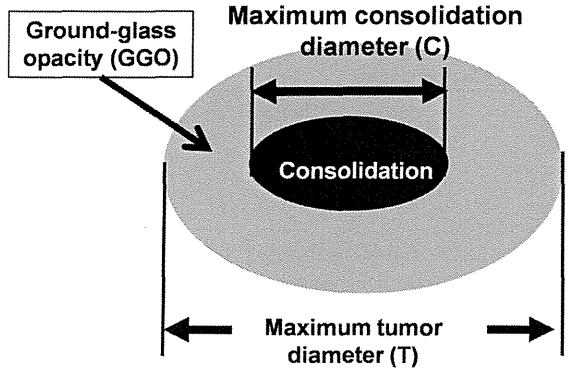

FIGURE 1. Calculation of the C/T ratio to define radiologic noninvasive lung cancer on TSCT. The maximum diameter of consolidation (C) is divided by the maximum tumor diameter (T) to give the C/T ratio. *GGO*, Ground-glass opacity; *C/T*, consolidation/tumor; *TSCT*, thin-section computed tomography.

analyses were performed with SAS software release 9.2 (SAS Institute, Inc, Cary, NC) by the JCOG Data Center.

RESULTS

Prognosis of Patients According to a Consolidation/Tumor Ratio 0.5 or Less (n = 545)

The patient characteristics of the entire cT1a-b (≤3.0 cm) population are shown in Table 1. Among the 545 patients, 233 (42.8%) were men and 312 (57.2%) were women. The median age was 62 years (range, 35-75 years). The median follow-up period among overall patients was 7.1 years after surgery (range, 0-8.6 years). The overall and relapse-free survival curves of the cT1a-b (≤3.0 cm) population are presented in Figure 2, and the 5-year overall and relapse-free survivals were 90.6% and 84.7%, respectively. Among the 545 adenocarcinomas, when a C/T ratio 0.5 or less in cT1a-b (≤3.0 cm) was used as a radiologic criterion for noninvasive cancer, 121 adenocarcinomas (22.2%) were diagnosed as noninvasive and 424 adenocarcinomas (77.8%) were diagnosed as invasive. Among the 121 radiologic noninvasive adenocarcinomas, 115 (95.0%) were precisely determined to be pathologic noninvasive cancer. Among the 424 radiologic invasive adenocarcinomas, 161 (38.0%) were precisely determined to be pathologic invasive cancer. Therefore, the specificity and sensitivity were 96.4% (95% confidence interval [CI], 92.3-98.7) and 30.4% (95% CI, 25.8-35.3), respectively. The overall survival curves for radiologic noninvasive cancer (n = 121) and invasive cancer (n = 424) are presented in Figure 3, *A*. The 5-year overall survivals for noninvasive cancer and invasive cancer were 96.7% and 88.9%, respectively, and the difference in overall survival was statistically significant ($P < .001$). The relapse-free survival curves for radiologic noninvasive cancer (n = 121) and invasive cancer (n = 424) are presented in Figure 3, *B*. The 5-year relapse-free survivals for noninvasive and invasive cancer were 95.9% and 81.6%, respectively, and the difference in relapse-free survival was statistically significant ($P < .001$).

TABLE 1. Patient characteristics of the entire cT1a-b (≤3.0 cm) population (n = 545)

Characteristics	No. of patients (%)
Gender	
Men	233 (42.8)
Women	312 (57.2)
Age (y)	
Median (range)	62 (35-75)
Maximum tumor diameter on TSCT*	
≤2.0 cm	289 (53.0)
>2.0-3.0	254 (46.6)
Unknown	2 (0.4)
C/T ratio on TSCT* and final pathology†	
≤0.5 (radiologically noninvasive)	121 (22.2)
Pathologically noninvasive	115 (21.1)
Pathologically invasive	6 (1.1)
>0.5 (radiologically invasive)	424 (77.8)
Pathologically noninvasive	263 (48.3)
Pathologically invasive	161 (29.5)

TSCT, Thin-section computed tomography; *C/T*, consolidation/tumor. *Maximum tumor diameter and a C/T ratio on TSCT were both evaluated by a central radiologic review board. †Patients with adenocarcinoma that was diagnosed at the time of surgery were eligible, and 16 patients with a different final pathologic diagnosis were included in the pathologically invasive group.

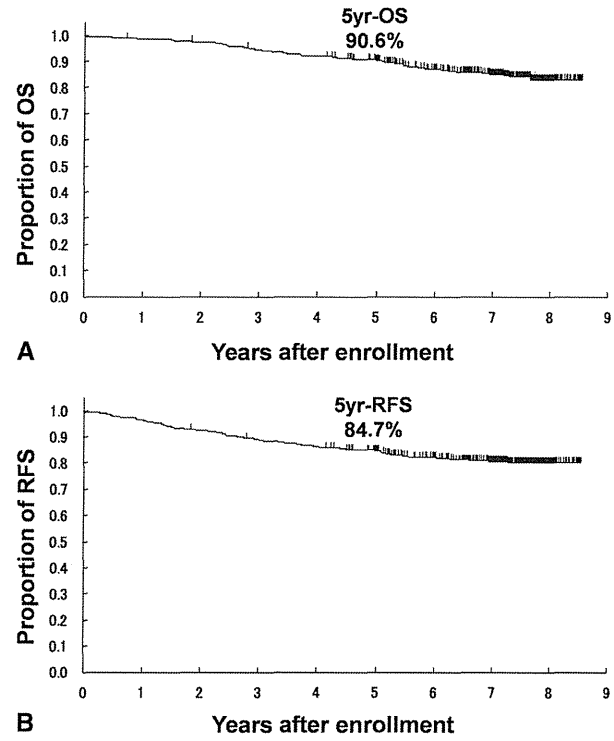

FIGURE 2. Overall (A) and relapse-free (B) survival curves for the entire group (cT1a-b, ≤3.0 cm, n = 545). *OS*, Overall survival; *RFS*, relapse-free survival.

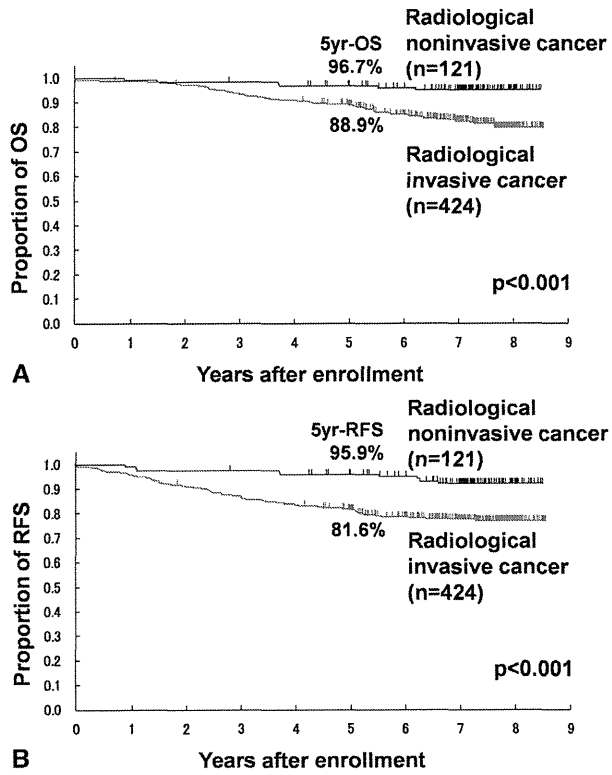

FIGURE 3. Overall (A) and relapse-free (B) survival curves for radiologically noninvasive (n = 121) and invasive (n = 424) adenocarcinomas based on a C/T ratio of 0.50 or less in cT1a-b (≤3.0 cm) for noninvasiveness on TSCT. The differences in overall and relapse-free survival are statistically significant ($P < .001$ and $< .001$, respectively). *OS*, Overall survival; *RFS*, relapse-free survival; *C/T*, consolidation/tumor; *TSCT*, thin-section computed tomography.

Prognosis of Patients According to a Consolidation/Tumor Ratio 0.25 or Less for cT1a Adenocarcinomas (≤2.0 cm in Size) (n = 289)

The patient characteristics of this population are shown in Table 2. Among the 289 patients, 129 (44.6%) were men and 160 (55.4%) were women. The median age was 61 years (range, 35-75 years). The median follow-up period among overall patients was 7.1 years after surgery (range, 0-8.5 years). The overall and relapse-free survival curves for the cT1a (≤2.0 cm) population (n = 289) are presented in Figure 4, and the 5-year overall and relapse-free survivals were 93.0% and 88.9%, respectively. Among the 289 cT1a (≤2.0 cm) adenocarcinomas, when a C/T ratio 0.25 or less was used as a radiologic criterion for noninvasive cancer, 35 adenocarcinomas (12.1%) were diagnosed as noninvasive and 254 adenocarcinomas (87.9%) were diagnosed as invasive. Among the 35 radiologic noninvasive adenocarcinomas, 34 (97.1%) were precisely determined to be pathologic noninvasive cancer. Among the 254 radiologic invasive adenocarcinomas, 78 (30.7%) were precisely determined to be pathologic invasive cancer. Therefore, the specificity and sensitivity were 98.7% (95% CI, 93.2-100.0) and 16.2% (95% CI, 11.5-21.9), respectively. The overall survival curves for radiologic noninvasive cancer (n = 35) and invasive cancer (n = 254) are presented in Figure 5, *A*. The 5-year overall survivals for noninvasive cancer and invasive cancer were 97.1% and 92.4%, respectively, and the difference in overall survival was not statistically significant ($P = .259$). The relapse-free survival curves for radiologic noninvasive cancer (n = 35) and invasive cancer (n = 254) are presented in Figure 5, *B*. The 5-year relapse-free survivals for noninvasive and invasive cancer were 97.1% and 87.7%, respectively, and the difference in relapse-free survival was not statistically significant ($P = .106$).

TABLE 2. Patient characteristics of cT1a (≤2.0 cm) population (n = 289)

Characteristics	No. of patients (%)
Gender	
Men	129 (44.6)
Women	160 (55.4)
Age (y)	
Median (range)	61 (35-75)
C/T ratio on TSCT* and final pathology†	
≤0.25 (radiologically noninvasive)	35 (12.1)
Pathologically noninvasive	34 (11.8)
Pathologically invasive	1 (0.3)
>0.25 (radiologically invasive)	254 (87.9)
Pathologically noninvasive	176 (60.9)
Pathologically invasive	78 (27.0)

C/T, Consolidation/tumor; *TSCT*, thin-section computed tomography. *Maximum tumor diameter and a C/T ratio on TSCT were both evaluated by a central radiologic review board. †Patients with adenocarcinoma that was diagnosed at the time of surgery were eligible, and the patients with a different final pathologic diagnosis were included in the pathologically invasive group.

Prognosis of Patients With cT1b Adenocarcinomas (>2.0-3.0 cm in Size) (n = 254) According to a Consolidation/Tumor Ratio 0.5 or Less

Two patients with unknown preoperative tumor size were excluded. Among the 254 patients, 104 (40.9%) were men and 150 (59.1%) were women. The median age was 62 years (range, 37-75 years). The median follow-up period among overall patients was 7.0 years after surgery (range, 0-8.5 years). The overall and relapse-free survivals for the cT1b (>2.0-3.0 cm) population were 87.8% and 79.9%, respectively. When a C/T ratio 0.5 or less was used as a radiologic criterion for noninvasive cancer, 54 adenocarcinomas (21.3%) were diagnosed as noninvasive and 200 adenocarcinomas (78.7%) were diagnosed as invasive. The overall survival curves for radiologic noninvasive cancer (n = 54) and invasive cancer (n = 200) are presented in Figure 6, *A*. The 5-year overall survivals for noninvasive cancer and invasive cancer were 96.3% and 85.5%, respectively, and the difference in overall survival was statistically significant

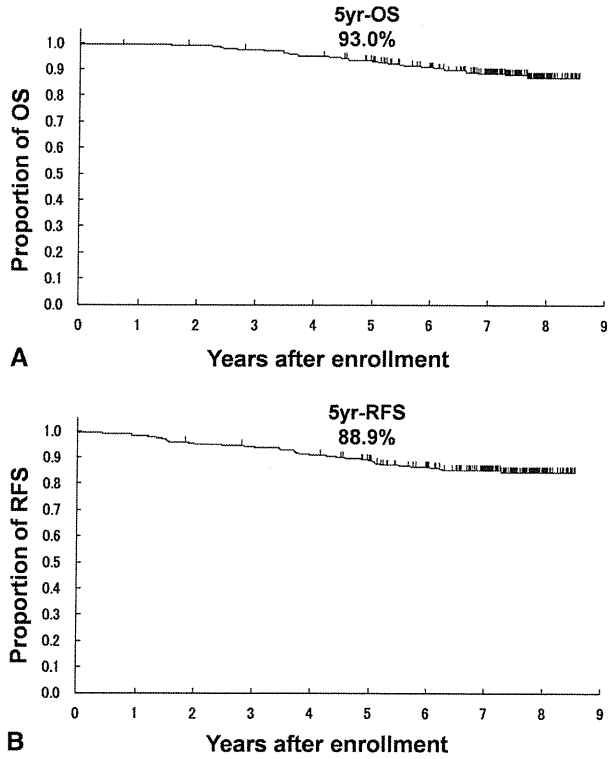

FIGURE 4. Overall (A) and relapse-free (B) survival curves for the cT1a (≤2.0 cm) group (n = 289). *OS*, Overall survival; *RFS*, relapse-free survival.

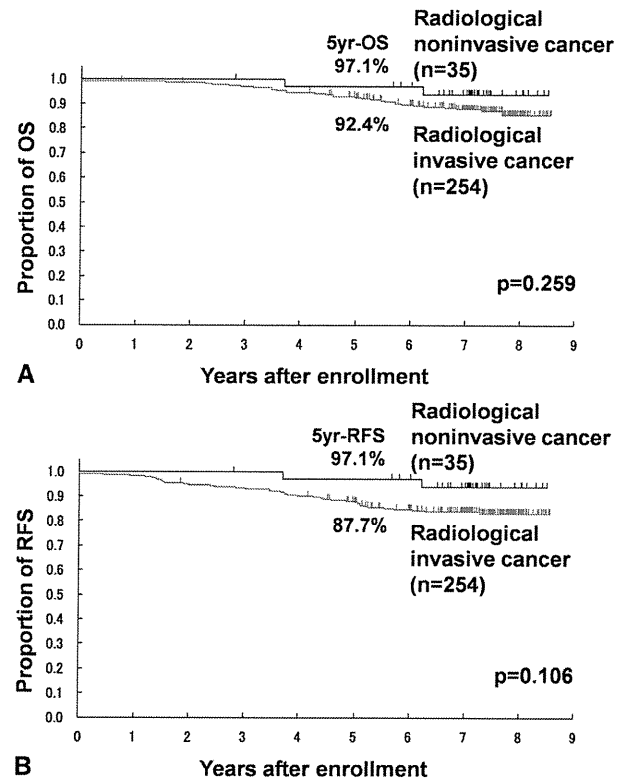

FIGURE 5. Overall (A) and relapse-free (B) survival curves for radiologically noninvasive (n = 35) and invasive (n = 254) adenocarcinomas based on a C/T ratio of 0.25 or less in cT1a (≤2.0 cm) for noninvasiveness on TSCT. The differences in overall and relapse-free survival are not statistically significant (P = .259 and .106, respectively). *OS*, Overall survival; *RFS*, relapse-free survival; *C/T*, consolidation/tumor; *TSCT*, thin-section computed tomography.

(P = .003). The relapse-free survival curves for radiologic noninvasive cancer (n = 54) and invasive cancer (n = 200) are presented in Figure 6, *B*. The 5-year relapse-free survivals for noninvasive and invasive cancer were 94.4% and 76.0%, respectively, and the difference in relapse-free survival was statistically significant (P = .003).

DISCUSSION

During the past 80 years, the surgical mode of pulmonary resection for lung cancer has evolved from pneumonectomy to lobectomy.[10,11] Currently, resection of the entire tumor-bearing lobe is being adopted as the standard mode of surgical resection for lung cancer. During this period, attempts have been made to minimize the resection through lobectomy to limited sublobar resection. Most importantly, the North American Lung Cancer Study Group conducted a prospective, randomized trial that compared limited resection with lobectomy for stage I lung cancer.[12] The principal finding in that study was a 3-fold increase in local recurrence (17.2% vs 6.4%) in patients who had sublobar resection and a 2.4-fold increase in those with segmental resection. A 30% increase in the overall death rate and a 50% increase in the rate of death with cancer in patients with limited resection were also observed compared with those with lobectomy. Therefore, it was concluded that lobectomy should still be considered the surgical procedure of choice for peripheral T1N0 non–small cell lung cancer. However, present-day critiques of this study are arising with regard to the marginal prognostic significance, poor preoperative workup for metastasis, slow accrual rate of the study, absence of data on pulmonary function (no demonstration of superiority in pulmonary function for lesser resection), and the notion that this study is outdated.

Despite the results of the North American Lung Cancer Study Group study, many nonrandomized studies have been published, and their results suggested that an equivalent prognosis could be achieved with limited sublobar resection for selected non–small cell lung cancer as with lobectomy.[13-18] Especially for earlier forms of noninvasive or minimally invasive adenocarcinoma such as bronchioloalveolar carcinoma, it has been shown that these cases present with a GGO appearance on TSCT and could be treated with limited sublobar resections, such as wide wedge resection and segmentectomy.[13-18] Because of the accumulation of excellent prognoses by limited resection in nonrandomized studies, surgeons require a fair comparison of lobectomy

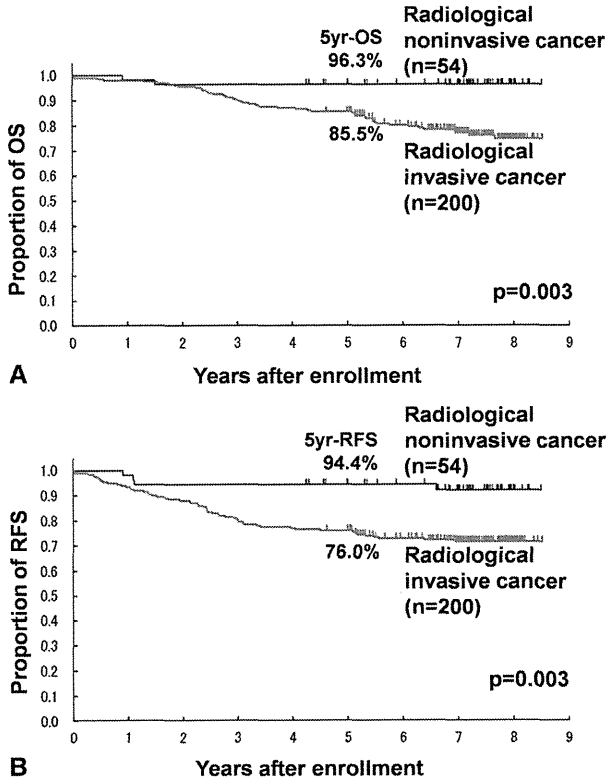

FIGURE 6. Overall (A) and relapse-free (B) survival curves for radiologically noninvasive (n = 54) and invasive (n = 200) adenocarcinomas based on a C/T ratio ≤0.5 in cT1b (>2.0-3.0 cm) for noninvasiveness on TSCT. The differences in overall and relapse-free survival are statistically significant (P = .003 and .003, respectively). *OS*, Overall survival; *RFS*, relapse-free survival; *C/T*, consolidation/tumor; *TSCT*, thin-section computed tomography.

and limited resection according to appropriate radiologic criteria for patient selection.

The radiologic criteria for noninvasive adenocarcinoma need to be evaluated by both pathologic and prognostic aspects. The radiology-pathology correlation has been studied in detail in JCOG0201, and the results have been published.[8] Briefly, JCOG 0201 failed to demonstrate that a C/T ratio 0.5 or less in T1a-b is precisely predictive of pathologic noninvasiveness. The specificity and sensitivity for these criteria were 96.4% (95% CI, 92.3-98.7) and 30.4% (95% CI, 25.8-35.3), respectively, and they did not reach the hypotheses in the JCOG 0201 study that the lower limit for 95% CI of specificity exceeds 97%. However, according to an exploratory study, a C/T ratio 0.25 or less in cT1a (≤2.0 cm) showed a specificity of 98.7% and a sensitivity of 16.2%, which was more precise for indicating noninvasive pathologic features. On the basis of these results, we recognized that a C/T ratio 0.25 or less in cT1a (≤2.0 cm) on TSCT is a better indicator of a noninvasive histology as early adenocarcinoma than a C/T ratio 0.50 or less in cT1a-b (≤3.0 cm). In this additional study of JCOG 0201, we looked at the prognoses obtained by these 2 radiologic criteria and focused on whether noninvasive adenocarcinomas defined by these 2 criteria could indicate a good prognosis as early adenocarcinoma.

In contrast to the results regarding the radiology-pathology correlation in the JCOG 0201 study, superb prognoses were obtained for noninvasive adenocarcinomas according to both of these criteria: The 5-year overall survivals for a C/T ratio 0.50 or less in cT1a-b (≤3.0 cm) and a C/T ratio 0.25 or less in cT1a (≤2.0 cm) were 96.7% and 97.1%, respectively. Although our previous report stated that a C/T ratio 0.25 or less in cT1a (≤2.0 cm) was an appropriate indicator to select patients with noninvasive lung adenocarcinomas suitable for limited sublobar resection,[8] these results indicate that even with a generous radiologic criterion on TSCT (a C/T ratio ≤0.50 in cT1a-b), we can precisely select a group of patients with early adenocarcinomas who have an excellent prognosis. For these patients, it is considered that limited sublobar resection achieves a prognosis that is almost the same as that with lobectomy and a better preservation of the lung parenchyma. In terms of the postoperative prognosis, this analysis indicates that noninvasive adenocarcinomas can be properly selected on TSCT using radiologic criteria of a C/T ratio of 0.50 or 0.25.

There is a caution regarding the excellent prognoses of the patients with noninvasive adenocarcinomas in this study. Patients included in the JCOG 0201 study underwent lobectomy and hilar/mediastinal lymph node dissection, which is usually indicated for any type of resectable lung cancer as a radical resection. The prognoses in this study were achieved by such radical resection; therefore, the same prognoses are not warranted for these tumors if they are resected with limited sublobar resections, although this was suggested by previous studies with case series.[13-18] We should still be prudent in addressing the excellent prognoses of early adenocarcinomas with surgical resection other than lobectomy.

On the basis of these results, 2 prospective, collaborative studies between JCOG and the West Japan Oncology Group, JCOG0804/WJOG4507L (UMIN-CTR [www.umin.ac.jp/ctr/] No. UMIN000002008) and JCOG0802/WJOG4607L (UMIN-CTR No.UMIN000002317), are under way on peripherally located adenocarcinomas of the lung.[19] In these studies, a radiologic criterion of a C/T ratio 0.25 or less in cT1a is used to define noninvasive adenocarcinomas. For radiologic noninvasive adenocarcinomas with a tumor diameter of 2 cm or less, a phase II study with 1 experimental arm (JCOG0804/WJOG4507L) is under way in which tumors are resected with a wide wedge resection. As of March 2012, the target number of accrual (330 patients) was reached, and maturation of follow-up data on recurrence and prognosis is awaited. For radiologic invasive adenocarcinomas with a tumor diameter 2.0 cm or less and

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a C/T ratio greater than 0.25, a prospective, randomized phase III study (JCOG0802/WJOG4607L) between lobectomy and segmentectomy in a noninferiority setting is under way. The primary and key secondary end points are overall survival and postoperative pulmonary function, respectively. If the prognosis of those undergoing segmentectomy is not inferior to that of those undergoing lobectomy and the pulmonary function of those undergoing segmentectomy is significantly better than that of those undergoing lobectomy, we will conclude that segmentectomy should be the standard mode of pulmonary resection for a peripherally located radiologic invasive adenocarcinoma with a diameter of 2.0 cm or less and a C/T ratio greater than 0.25. The target number of patients is 1100, and accrual is under way. This study will clarify the pathologic and survival outcomes of the patients with cT1a (≤2.0 cm) tumor and a C/T ratio ranging from 0.25 to 0.5, and might validate the radiologic definition of a C/T ratio 0.5 or less for noninvasive lung adenocarcinoma among the cT1a (≤2.0 cm) group. In North America, a similar study entitled Cancer and Leukemia Group B 140503 is also under way, in which the prognosis and preservation of pulmonary function is compared between lobectomy and limited resection in a noninferiority setting.[20,21] The current survival analysis showed that noninvasive adenocarcinomas indicative of limited resection could be selected by the size of 3 cm or less and a C/T ratio 0.5 or less. Even the patients with cT1b tumors (>2-3 cm) may be candidates for limited resection if their preoperative C/T ratio is 0.5 or less. For cT1b tumors, segmentectomy but not wide wedge resection should be considered as an appropriate limited resection to obtain adequate surgical margins. Future prospective study will be needed to investigate the clinical significance of intentional segmentectomy for such a population.

CONCLUSIONS

Despite the finding that a noninvasive pathology is better predicted with a C/T ratio 0.25 or less on TSCT in cT1a (≤2.0 cm) than with 0.50 or less in cT1a-b (≤3.0 cm), both of these radiologic criteria could identify a group of patients with an excellent prognosis, with a 5-year overall survival of approximately 97%. These criteria can be used to select patients with peripherally located adenocarcinomas in whom a limited resection, such as wide wedge resection or segmentectomy, might be safely indicated.

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Gene expression profiling and molecular pathway analysis for the identification of early-stage lung adenocarcinoma patients at risk for early recurrence

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Abstract. Clinicohistopathological staging is insufficient to predict disease progression and clinical outcome in lung carcinoma. Based on the results of the principal component analysis of 24 samples of early-stage lung adenocarcinoma, two subgroups were identified within the early-relapse group. The histological classification of all samples of group A was poorly differentiated, whereas one out of three in group B was poorly differentiated. DAVID functional annotation analysis revealed that the molecular pathways enriched in group A included those associated with cell adhesion molecules (CAMs), cell cycle and antigen processing and presentation, whereas those in group B included CAMs, T cell receptor signaling, cytokine-cytokine receptor interaction, toll-like receptor signaling, chemokine signaling, primary immunodeficiency and natural killer cell-mediated cytotoxicity. The CAM pathway was enriched in both groups. This comprehensive gene expression and functional pathway analysis identified a distinct molecular pathway, CAMs, that correlated with the early relapse of patients with early-stage lung adenocarcinoma.

Introduction

Lung cancer is the leading cause of cancer-related deaths in Japan and also worldwide in most developed countries. Every year, ~60,000 individuals succumb to lung cancer in Japan, and the number is increasing rapidly. Even in early-stage lung cancer, ~40% of patients with stage I and II non-small cell lung cancer (NSCLC) die from recurrent disease within 5 years despite complete resection (1,2). The precise diagnosis and classification of cancers are critical for the selection of appropriate therapies. However, since no reliable clinical or molecular predictors are currently available, it is difficult to select high-risk patients who require more aggressive therapies such as adjuvant chemotherapy.

Genetic abnormalities that exist in a certain population of early-stage lung cancer patients possibly induce aggressive phenotypes that demonstrate rapid tumor growth, persistent invasiveness and a high potential for distant metastasis. The expression of a number of genes is altered in cancer cells due to mutations, deletions, amplifications, and either the upregulation or downregulation of mRNA transcription. Comprehensive DNA microarray analysis of gene expression patterns is a powerful tool that permits the simultaneous evaluation of a large number of genes in cancer cells (3,4). Microarray gene expression profiling has recently been used to define prognostic signatures in patients with NSCLC (5-11). However, information concerning gene expression profiling and molecular pathways relating to the outcomes of patients with early-stage lung cancer has yet to be well characterized.

Adenocarcinoma is currently the predominant histological subtype of NSCLC. The results of several expression profiling studies have demonstrated that the expression profiles are distinctive and recapitulate the known histological subtypes (5-7). As a significant proportion of patients relapse within 2 years, identification of early-stage patients with a poor prognosis could delineate the appropriate candidates for adjuvant therapy. The present study aimed to identify a novel prognostic signature in early-stage lung adenocarcinoma using cDNA microarray and bioinformatics analysis.

Materials and methods

Patient samples. Intraoperatively, immediately upon removal of a lung lobe in which a primary lung carcinoma was located, a 500-mg sample of tumor tissue was cut and immediately immersed in liquid nitrogen and stored at -80°C until use, as previously reported (12). We studied frozen specimens of lung cancer tissue from 64 randomly selected patients who underwent complete resection of stage I or II NSCLC lesions

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at Tokyo Medical University, Tokyo, Japan from May 2003 to December 2006. Tumor tissues were processed by the Human Tissue Bank section at our department according to standard operating procedures and protocols. Briefly, frozen tissue samples at -80°C were pulverized, and total cellular RNA was collected from each flash-frozen sample using TRIzol RNA isolation reagent (Invitrogen). Total RNA was processed with an RNeasy Mini kit (Qiagen). *In vitro* transcription-based RNA amplification was then performed on at least 8 μg of total RNA from each sample. The RNA quality was assessed using a bioanalyzer (model 2100, Agilent). According to the results from the RNA quality assay, 24 lung adenocarcinoma samples were selected as our dataset.

Microarray analysis. Complementary DNA was synthesized using the T7-(dT)24 primer: 59-GGCCAGTGAATTGTA ATACGACTCACTATAGGGAGGCGG-(dT)24-39. The cDNA was processed using phase-lock gel phenol/chloroform extraction (#E0032005101, Fisher). Next, *in vitro* transcriptional labeling with biotin was performed using the Enzo BioArray kit (#900182, Affymetrix). The resulting cRNA was processed again using the RNeasy Mini kit. Labeled cRNA was hybridized to an Affymetrix GeneChip (Human Genome-133 Plus 2.0 Array) according to the manufacturer's instructions. The raw fluorescence intensity data within the CEL files were preprocessed with the robust multichip average algorithm, as implemented with the R packages from Bioconductor. This algorithm analyzes the microarray data in three steps: a background adjustment, quantile normalization, and finally summation of the probe intensities for each probe set using a log scale linear additive model for the log transform of (background corrected, normalized) PM intensities.

Data analysis. Affymetrix Human Genome-U133 Plus 2.0 GeneChip data, quantified with MAS5, were imported into the Subio Platform (Subio Inc., Tokyo, Japan). Signals <1 were replaced with 1, log2 transformed, and then mean-subtracted by each probe set to obtain the log ratio against the average of the expression patterns. No normalization was applied.

Samples were classified into two groups, recurrence-positive and recurrence-negative. Probe sets in both groups whose detection values were absent in half of the samples were removed. At this point, 28674 out of 54682 probe sets remained. Finally, unvarying probe sets, whose log ratios were between -1 and +1 in all samples, were filtered out to obtain the final quality controlled probe sets (24420).

Principal component analysis (PCA) was applied to the log ratio data of quality controlled genes. We recognized that the samples in the recurrence-positive group might be distinguishable as PC1 score negative (A) and positive (B) subgroups.

We extracted the differentially expressed genes (DEGs) for both A and B subgroups. We defined DEGs for A as being >4-fold upregulated or downregulated compared with the average of the recurrence-negative group, and having Mann-Whitney U-test P-values of <0.05 between the recurrence-negative group and the recurrence-positive A subgroup. A total of 721 probe sets were selected as DEG for A. Similarly, we obtained 274 probe sets as DEGs for B, which showed a >2-fold change and P-values of <0.05 by the Mann-Whitney U-test, as compared with the recurrence-negative group.

Biological analysis of the DEG lists. We searched 171 and 33 enriched GO terms for DEGs determined for the A and B group, respectively, with the annotation analysis plug-in of the Subio platform (data not shown). We further analyzed these lists with the DAVID functional annotation web tool (http://david.abcc.ncifcrf.gov) and obtained the lists of enriched KEGG pathways (Tables I and II).

Ethical considerations. Written informed consent was obtained from the patients for tissue procurement prior to surgery and their medical records were maintained according to protocols approved by the Institutional Review Board of Tokyo Medical University (no. 965).

Results

Patient information. As shown in Table I, there were 14 male and 10 female patients enrolled in this study. The mean age was 65.3 years (range, 42-76). The histological classifications were all adenocarcinoma; 14 were well/moderately differentiated and 10 were poorly differentiated. The distribution of clinical staging demonstrated that most of the patients were early-stage IAB cases. Histological differentiation was significantly correlated with early recurrence (P=0.026), whereas no significant correlations were found among pathological stages IA, IB and IIA (P=0.061).

Correlation of patient outcome with putative adenocarcinoma classes. We aimed to ascertain whether lung cancer patient outcome correlates with the subclasses of lung adenocarcinomas defined herein. Based on the results of PCA of this series, two adenocarcinoma subgroups were identified within the early-relapse group of early-stage adenocarcinoma cases, which differentially expressed a broad range of gene patterns (Fig. 1).

Statistical analysis of the microarray data, when compared with the non-early-relapse group C, revealed 723 genes with significant differences in expression in the samples of group A, whereas 274 genes showed significant differences in expression in samples of group B. We searched 171 and 33 altered GO terms for DEGs in the A and B lists, respectively, with the annotation analysis plug-in of the Subio platform (data not shown).

The histological classification of all samples of group A was poorly differentiated, whereas only one out of three cases in group B was classified as poorly differentiated. In this series of early-stage IA-IIA adenocarcinomas, no papillary or bronchio-alveolar carcinoma subtypes were associated with recurrence within 2 years after complete resection.

Biological function analysis. Tables II and III document the 16 and 17 enriched pathways in groups A and B, respectively. Clusters of genes related to oncological or immunological functional signaling were found enriched in group A as were pathways such as cell adhesion molecules (CAMs), cell cycle, and antigen processing and presentation. In group B, the pathways included CAMs, T cell receptor signaling, cytokine-cytokine receptor interaction, toll-like receptor signaling, chemokine signaling pathway, primary immunodeficiency and natural killer cell mediated cytotoxicity. The CAM pathway was found to be enriched in both groups A and B.

Table I. Patient information regarding the 24 adenocarcinoma samples.

Variables	Total samples	Early recurrence		P-value
		Positive (n=8)	Negative (n=16)	
Median age (range)	65.3 (42-76)	67.0 (55-76)	64.5 (42-76)	0.551
Gender				0.143
Male	14	3	11	
Female	10	5	5	
Histological differentiation				0.026[a]
Well/moderate	14	2	11	
Poor	10	6	4	
p-Stage				0.061
IA	14	2	12	
IB	8	5	3	
IIA	2	1	1	

[a]Significant difference.

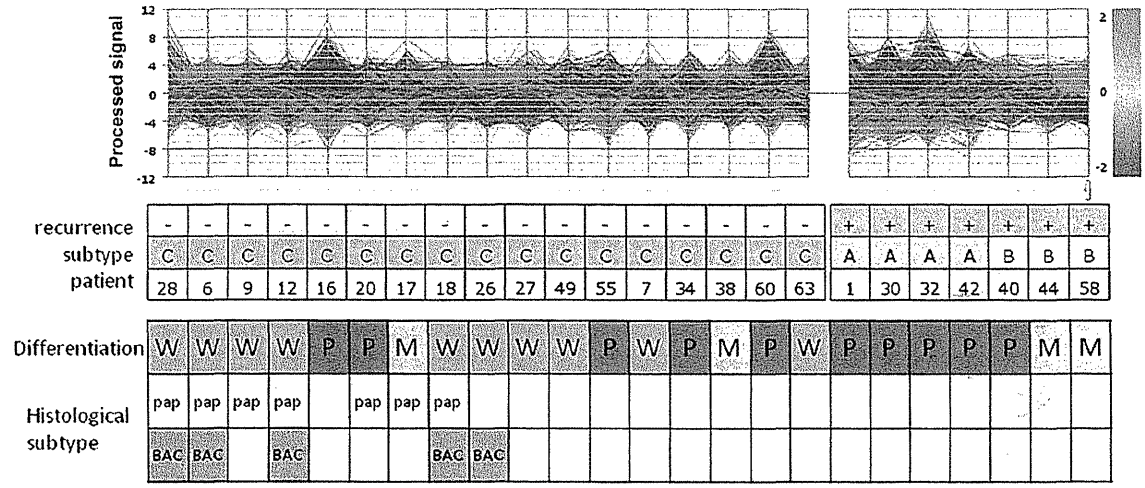

Figure 1. Principal component analysis correlated with differentiation and histological subtype. W, well differentiated; M, moderately differentiated; P, poorly differentiated; BAC, bronchio-alveolar carcinoma; pap, papillary carcinoma.

Discussion

The development of microarray technologies has made it possible to quantitate the expression of many thousands of genes simultaneously in a given sample (3,4). Comprehensive analysis of gene expression patterns in individual tumors should, therefore, provide detailed molecular portraits that can facilitate tumor classification. Several expression profiling studies concluded that expression profiles are distinctive and recapitulate known histological subtypes (5-7).

Genomic methods offer promise for the classification of human lung carcinomas. In one previous study, it is important to note that the performance of the adenocarcinoma classifier showed a better predictive accuracy than the squamous cell lung carcinoma (SCC) classifier (adenocarcinoma AUC = 0.83, SCC AUC = 0.68). This could have been due to the heterogeneity of the SCC samples as indicated by the two distinct subgroups showing differing clinical outcomes in this tumor type (9). Multiple independent studies of mRNA expression profiles in lung adenocarcinoma have proven highly reproducible. Analyses of the relationship between expression profiles and tumor development and differentiation, the presence or absence of specific pathogenic mutations, patient prognosis and survival after surgical treatment, and specific histopathology all appear to be promising (13).

Adenocarcinoma is currently the predominant histological subtype of NSCLC. NSCLC composes the majority of bronchogenic carcinoma cases with a lesser fraction being small-cell lung carcinomas. The three main subtypes of NSCLC are adenocarcinoma (60%), SCC (25%) and large-cell cancer (5%). Adenocarcinoma has replaced SCC as the most frequent histological subtype over the last 25 years (1,2,14). Therefore,

Table II. Enriched pathways in group A.

Category	Term	Count	%	P-value	Benjamini
KEGG_PATHWAY	Asthma	8	1.5	0.000019	0.0024
KEGG_PATHWAY	Allograft rejection	8	1.5	0.000085	0.0053
KEGG_PATHWAY	Graft-versus-host disease	8	1.5	0.00014	0.0061
KEGG_PATHWAY	Cell adhesion molecules (CAMs)	14	2.5	0.00017	0.0052
KEGG_PATHWAY	Type I diabetes mellitus	8	1.5	0.00023	0.0059
KEGG_PATHWAY	Intestinal immune network for IgA production	8	1.5	0.00062	0.013
KEGG_PATHWAY	Autoimmune thyroid disease	8	1.5	0.0008	0.014
KEGG_PATHWAY	Hematopoietic cell lineage	10	1.8	0.0011	0.017
KEGG_PATHWAY	Systemic lupus erythematosus	10	1.8	0.003	0.041
KEGG_PATHWAY	Antigen processing and presentation	8	1.5	0.013	0.15
KEGG_PATHWAY	PPAR signaling pathway	7	1.3	0.018	0.19
KEGG_PATHWAY	Oocyte meiosis	9	1.6	0.018	0.18
KEGG_PATHWAY	Viral myocarditis	7	1.3	0.02	0.18
KEGG_PATHWAY	Arachidonic acid metabolism	6	1.1	0.027	0.22
KEGG_PATHWAY	Cell cycle	9	1.6	0.036	0.27
KEGG_PATHWAY	Drug metabolism	6	1.1	0.04	0.27

Table III. Enriched pathways in group B.

Category	Term	Count	%	P-value	Benjamini
KEGG_PATHWAY	T cell receptor signaling pathway	11	4.8	0.0000046	0.00048
KEGG_PATHWAY	Cytokine-cytokine receptor interaction	16	7	0.0000075	0.00039
KEGG_PATHWAY	Toll-like receptor signaling pathway	9	3.9	0.00014	0.0047
KEGG_PATHWAY	Cell adhesion molecules (CAMs)	10	4.3	0.00016	0.0042
KEGG_PATHWAY	Leukocyte transendothelial migration	8	3.5	0.0021	0.041
KEGG_PATHWAY	Chemokine signaling pathway	10	4.3	0.0021	0.035
KEGG_PATHWAY	Intestinal immune network for IgA production	5	2.2	0.0064	0.091
KEGG_PATHWAY	Autoimmune thyroid disease	5	2.2	0.0074	0.091
KEGG_PATHWAY	Primary immunodeficiency	4	1.7	0.016	0.17
KEGG_PATHWAY	Type I diabetes mellitus	4	1.7	0.026	0.24
KEGG_PATHWAY	Axon guidance	6	2.6	0.047	0.36
KEGG_PATHWAY	Cytosolic DNA-sensing pathway	4	1.7	0.052	0.37
KEGG_PATHWAY	Natural killer cell mediated cytotoxicity	6	2.6	0.053	0.35
KEGG_PATHWAY	NOD-like receptor signaling pathway	4	1.7	0.069	0.41
KEGG_PATHWAY	Focal adhesion	7	3	0.087	0.46
KEGG_PATHWAY	RIG-I-like receptor signaling pathway	4	1.7	0.095	0.47
KEGG_PATHWAY	Prion diseases	3	1.3	0.1	0.47

we focused on adenocarcinoma of the lung, and particularly whether we could identify a novel prognostic signature of early recurrence in early-stage lung adenocarcinoma using cDNA microarray techniques.

The data indicated that patterns of gene expression obtained from cDNA microarray studies of crudely dissected lung tumors can be used to detect tumor subtypes that correlate with biological and clinical phenotypes. Specifically, patterns of gene expression were found that corresponded to the major morphological classes of lung tumors. In addition, we were able to define two subgroups of early recurrence in the adenocarcinoma cases that differed not only in gene expression patterns, but also in clinical and pathological properties, including histological differentiation and subtype. In the statistical analysis of microarray data, when compared with the non-early-recurrence group C, we revealed 723 genes with significant differences in expression in the samples of group A, whereas 274 genes showed significant differences in

expression in group B. The differentially expressed genes were classified according to biological processes. We searched 171 and 33 enriched GO terms for DEGs for the A and B lists, respectively, with the annotation analysis plug-in of the Subio platform (data not shown).

Gene annotation enrichment analysis is a functional analysis technique that has gained widespread attention and for which many tools have been developed. The differentially expressed genes were classified according to biological processes and molecular functions using the functional annotation clustering tool of the DAVID bioinformatics resources. The DAVID functional clustering analysis revealed 16 significantly altered biological pathways in group A that included 3 distinct functionally related metastatic categories, specifically CAMs, cell cycle, and antigen processing and presentation. In group B, there were 17 significantly altered biological pathways, including 7 distinct functionally related metastatic categories. Notably, the CAM pathway was the most interrelated in both groups. In addition, the T cell receptor signaling pathway, cytokine-cytokine receptor interaction, toll-like receptor signaling pathway, chemokine signaling pathway, primary immunodeficiency and natural killer cell mediated cytotoxicity were also altered (Tables II and III). These results suggest that the possibility of metastasis of early-stage lung adenocarcinoma was closely related to the CAM pathway. Interestingly, considering the relationship between group A or group B and histological differentiation as poor or well/moderate, respectively, the metastatic possibility of poorly differentiated early adenocarcinoma appeared to be correlated with tumor development factors, such as the cell cycle, whereas that of well/moderately differentiated early-stage adenocarcinoma appeared to be correlated with host immunological factors, such as the T cell receptor signaling pathway, cytokine-cytokine receptor interaction, the toll-like receptor signaling pathway, the chemokine signaling pathway, primary immunodeficiency and natural killer cell mediated cytotoxicity.

Our results suggest that the particular genes that define the clusters and molecular pathways, or that are associated with early recurrence, likely reflect the characteristics of the particular tumors included in the analysis. Current therapy for patients with early-stage disease usually consists of surgical resection without adjuvant treatment. Clearly, the identification of a high-risk group among early-stage patients would lead to consideration of additional therapeutic interventions, possibly leading to improved survival of these patients.

To our knowledge, this is the first study utilizing cDNA microarray techniques, followed by molecular functional pathway analysis, concerning the early recurrence of early-stage adenocarcinoma of the lung. However, there were some limitations to this study. Firstly, this was a small data set analysis at a single institute. A large cohort sample of patients from multiple institutions is needed. Secondly, the potential interactions of the many specific individual genes and their clusters in lung tumor biology and clinical outcome exist. This may be due to the different platforms used (different genes analyzed) and the different algorithms for selecting functional categories. Thirdly, hierarchical clustering methods and functional analysis offer a powerful approach to class discovery, but provide no means of determining validity for the classes discovered. This is still a putative functional analysis. It is important to state that several *in vitro* and *in vivo* studies are still needed to demonstrate whether these mechanisms are effective in reality.

In conclusion, in the present study, we present a comprehensive gene expression analysis and functional pathway analysis of early-stage lung adenocarcinomas, wherein we identified a distinct molecular pathway category, the CAMs, which correlated with the early relapse of early-stage lung adenocarcinoma subclasses. Further *in vitro* and *in vivo* studies, which can demonstrate these mechanisms, are warranted.

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Early report

Micropapillary components in a lung adenocarcinoma predict stump recurrence 8 years after resection: A case report

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ABSTRACT

We report a rare case of lung adenocarcinoma in which micropapillary components were considered to cause stump recurrence. A woman in her fifties was diagnosed with lung cancer in the right middle lobe with invasion to the upper lobe, which was treated by a right middle lobectomy together with upper lobe partial resection. The cancer was pathologically diagnosed as adenocarcinoma and had a free surgical margin. There was no recurrence during the following 5 years and 8 months, and thus periodical surveillance, including computed tomography, was stopped. However, 2 years and 7 months after this, she was discovered to have an abnormal shadow on chest radiography, and a thorough examination revealed a 3-cm-sized tumor involving the previous surgical margin. Therefore, she underwent right upper lobectomy. We pathologically re-evaluated the first tumor and found that it was an adenocarcinoma with a micropapillary component in the periphery, 6 mm away from the surgical margin. In addition, a few tiny clusters of tumor cells were found to be floating within the alveolar spaces near the margin. The first and second tumors showed almost the same histological mixture of components of adenocarcinoma and the same EGFR mutation. From these results, we concluded the second tumor was a stump recurrence originating from the first tumor resection. This case illustrates the importance of careful pathological investigation when an autosuture instrument is used for a partial resection in a case of lung adenocarcinoma with micropapillary components. In such cases, it is particularly important to clarify if micropapillary components are floating near a stump.

1. Introduction

Generally, when a primary lung cancer invades another lobe, a combined lobectomy with partial resection is performed if a tumor is sufficiently distant from the stump of the resection. Despite this precaution, a stump recurrence sometimes occurs due to unknown causes. Primary lung cancers exhibit a variable histology and a high degree of pleomorphism. This complexity has recently been added to because the presence of micropapillary components consisting of floating tumor cells in alveolar spaces has been classified in the International Association for the Study of Lung Cancer/American Thoracic Society/European Respiratory Society International Multidisciplinary Classification of lung adenocarcinoma (New IASLC classification) [1]. This histological finding has been shown to associate with a poor prognosis [2–4]. However, relatively little is known about the relationship between this micropapillary component and stump recurrence. Here we report a case of stump recurrence of a primary nonmucinous adenocarcinoma, which may be closely associated with the micropapillary component identified during pathological and molecular investigation.

2. Case

A woman in her fifties was discovered to have an abnormal chest shadow on radiography, and computed tomography revealed a tumor in the right middle lobe of size 25 mm × 25 mm, with spiculation and invasion of the right upper lobe. Treatment consisted of a right middle lobectomy combined with an upper lobe partial resection. An intraoperative pathological examination for a surgical margin around the partially resected material was

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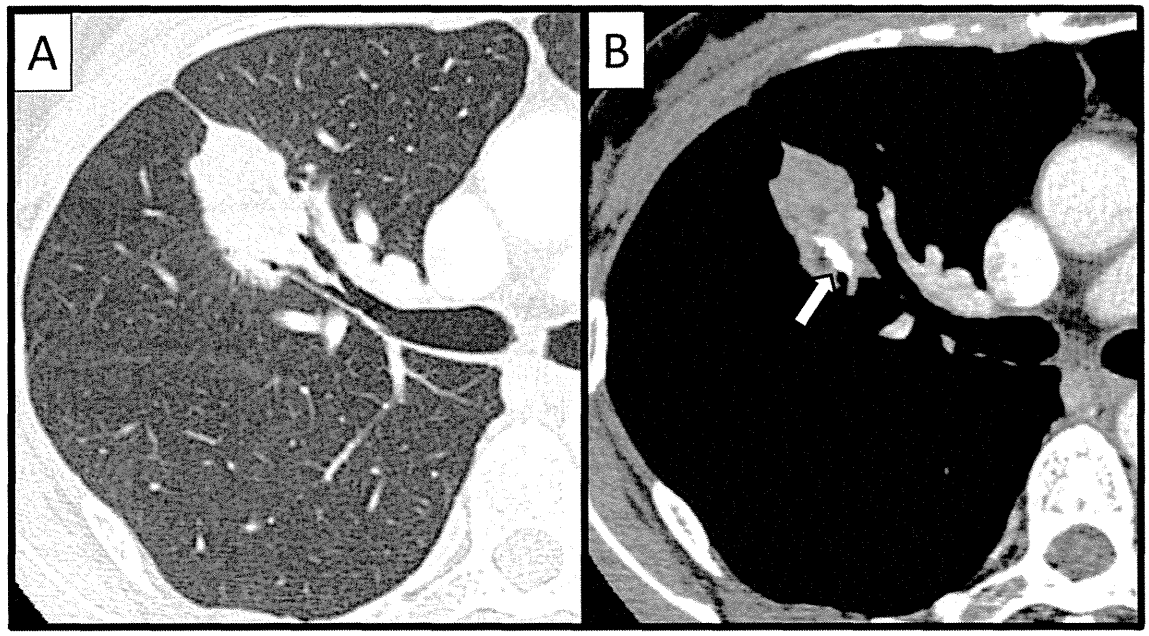

Fig. 1. Chest CT of the second tumor 8 years and 3 months after surgery (A: lung window and B: mediastinal window). The tumor involves the stapler line of the first operation at the right upper lobe (arrow).

not performed. Pathological analysis revealed the tumor to be an invasive adenocarcinoma, 26 mm × 19 mm × 23 mm in size, and predominantly composed of acinar tissue (50%), along with lepidic (15%), papillary (20%), micropapillary (10%), and solid tissues (5%). The tumor showed moderate lymph duct permeation with vascular invasion. We diagnosed patholohical T2aN0M0 lung adenocarcinoma in IB stage. She was followed up for 5 years and 8 months after surgery without adjuvant chemotherapy and showed no findings of recurrence; therefore, her routine follow-up was ended. However, 2 years and 7 months later, she again presented with an abnormal chest shadow on chest radiography, and medical examination and chest CT revealed a tumor in the right upper lobe involving a staple line of the first operation (Fig. 1). She was diagnosed with lung cancer and underwent a right upper lobectomy.

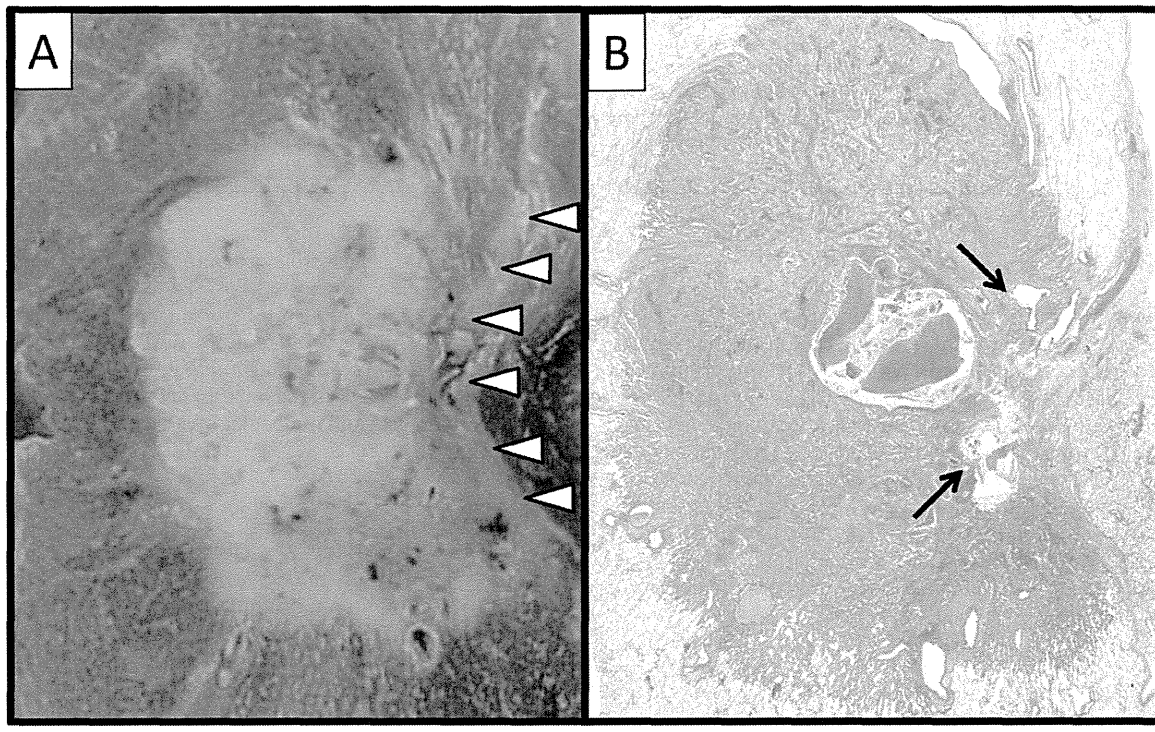

Fig. 2. The second tumor involved the previous surgical margin. Macroscopically, white arrowheads indicate the staple line of the last operation (A). Staples were removed during a specimen processing and the defects were microscopically left in the fibrotic line (B, black arrows).