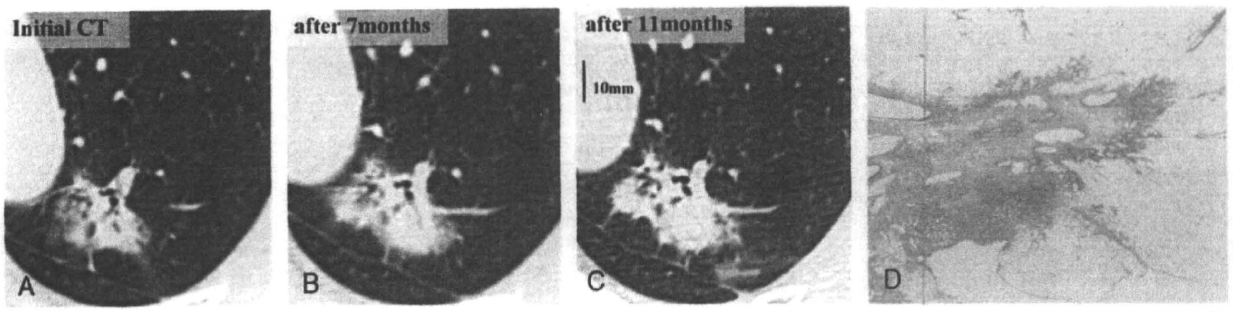

FIGURE 3. In a 68-year-old woman, a tumor was found in the left upper lobe. We first diagnosed this lesion as old inflammation. We observed this patient for a year and noted that her tumor grew very slowly and that the pleural indentations became more clearly defined. The TS-CT findings were as follows: The tumor was in the left upper lobe (S3). The tumor size was 35 × 24 mm. The tumor was polygonal with straight and concave margins. There were peripheral GGO areas around the tumor, dilated small bronchi within the tumor, and prominent pleural indentations. A, Initial image by TS-CT. The tumor in the left upper lobe was determined as an inflammatory lesion. B and C, Rescanned TS-CT images after 7 and 11 months more. The tumor increased slowly in diameter from 30 to 35 mm. The tumor in the left upper lobe is polygonal with straight and concave margins. Note the slight peripheral GGO around the tumor, the dilated air bronchograms in the tumor, and the prominent pleural indentation. D, This pathological specimen shows that most of the tumor area was occupied collapsed (80% of the tumor area) with a slight peripheral BAC. There were more than 3 ectatic small bronchi in the tumor area (pT2 N0 M0).

based on a modified Schwartz formula. The mean (SD) VDT was 1165 (773) days (Table 3).

Pathological Findings of BLA-Type Adenocarcinoma

The mean (SD) tumor size was 35.4 (7.3) mm (16–50 mm). All the 26 cases presented characteristic pathological findings as follows (Table 4): (1) There were extensive (a mean of 80%) areas of collapse within the tumors. (2) The peripheral areas of the tumors are bronchioloalveolar cell carcinoma (BAC), the area of which involved approximately 20% of the whole tumor area. (3) There were several dilated small bronchi within the collapse areas. Invasive lesions were observed only in 2 cases (4.4% and 8.8% of the whole tumor area). Lymphatic permeation and vascular invasion were observed in 1 and 3 cases, respectively. The pathological stages included IA in 8 cases, IB in 15, IIB in 2, and IIIA in 1. The World Health Organization pathological classification is BAC: 4 cases, mixed subtype with BAC; 6 cases, mixed subtype with BAC and papillary (or acinar); 16 cases.

EGFR and K-*ras* Mutation Analysis

EGFR mutations were observed in 17 (65.4%) of the 26 cases. The patients having tumors with *EGFR* mutations included 4 (of the 7) men and 13 (of the 19) women. Most (12 of 17, 70.5%) mutations detected were L858R point mutations in exon 21. The others (n = 5) were deletion mutations in exon 19. No K-*ras* mutations were found in any of the 26 cases (Table 5).

DISCUSSION

Some lung cancers are diagnosed as inflammatory lesions and are usually difficult to differentiate from inflammatory tumors.[11] Unfortunately, there have only been a few precise reports about such lung cancers.[12] Adenocarcinomas with BLA, a type of lung cancer, appear similar to inflammatory lesions. Our study focused on the precise characteristics of such cancers and how to differentiate BLA-type adenocarcinomas from inflammatory lesions.

All of our BLA-type lung adenocarcinoma CT findings are unique, with one pattern that included the same 4 characteristics including the following: (1) polygonal with straight and concave margins, (2) slight peripheral GGO, (3) 3 or more dilated small bronchi (air bronchograms), and (4) clear pleural indentation. Tumors with these characteristics are easily misdiagnosed as inflammatory lesions. Kohono et al[11] reported on CT appearances of focal organized pneumonia and concluded that oval masses with pleural tags along the bronchovascular bundle and on satellite lesions are suggestive of benign lesions; however, such CT findings vary greatly and are sometimes difficult to differentiate from bronchogenic carcinomas even on the most advanced CT equipment. They also reported on focal pneumonia with bronchial dilations, which appear similar to BLA-type adenocarcinomas.[5,11] Li et al[4] reported that polygonal tumors with smooth or semismooth margins are less frequently present in malignant lesions than in benign lesions. Because of the polygonal tumors with both straight and concave margins, BLA-type adenocarcinomas are sometimes overlooked as inflammatory lesions. In fact, in 9 of our cases, this type of adenocarcinoma was initially diagnosed as an old inflammatory lesion. The air bronchograms are reported to be useful CT findings that help us to differentiate adenocarcinomas from benign lesions.[13] However, it should be noted that air bronchograms are present in old inflammatory lesions in approximately 50% of such cases.[11] We tend to diagnose BLA-type adenocarcinoma findings that include ectatic bronchi as inflammatory bronchial ectasis. Polygonal lesions, including ectatic bronchiole and pleural tags, are also findings

TABLE 2. Characteristic TS-CT Findings of BLA

Polygonal with straight and concave margins
Slight peripheral GGO areas around the tumor
Three or more dilated air bronchograms within the tumor area
Clear pleural indentation

TABLE 3. Cases Followed up by CT

No. patients	9
Initial diagnosis	Inflammation (9 cases)
Observation time, d	758 (approximately 30–2555)
VDT, mean (SD), d	1165 (773)

TABLE 4. Pathological Findings of BLA Adenocarcinomas

Tumor diameter, mean (SD) [range], mm	35.4 (7.3) [16–50]
No. cases of peripheral BAC	26
Area of collapse, mean (range), %	80 (35–97)
Area of BAC, mean (range), %	19 (3–65)
Areas of invasive lesions, % (No. patients)	0 (15), 4.4 (1), and 8.8 (1)
Lymphatic permeation/vascular invasion	1/3
Pathological stage (IA/IB/IIB/IIIA)	8/15/2/1

in inflammatory lesions; however, tumors having slight periphery GGO areas are characteristic findings only observed in adenocarcinoma.[4] We have concluded that tumors with peripheral GGO areas are the most important difference between BLA-type adenocarcinomas and inflammatory lesions.

The pathological findings confirmed our diagnosis based on the CT findings. The characteristic pathological findings are as follows: (1) The peripheral GGO areas observed on the TS-CT scans correspond with the pathological findings of the BAC areas. The BAC parts occupied approximately 19% of the tumor areas. (2) The solid parts that can be seen on the TS-CT scans were pathologically confirmed to be areas of collapse. The collapsed parts occupied approximately 80% of the tumor area. (3) Three or more dilated small bronchus within the tumor area is a characteristic finding. We think that the growth mechanism of the BLA-type adenocarcinomas includes gradually expanding areas of BAC in periphery regions and extensive simultaneous collapse within the tumor area. The tumor shape becomes distorted, and pleural indentation become more clearly. The tissue around the small bronchi becomes distorted, and dilated small bronchi are formed.[5] In the 9 cases we observed over a period, the above-mentioned growth patterns were observed on the TS-CT scans (Fig. 3). In 1 of the 9 cases, the tumor temporally shrank because the inside of the tumor collapsed. The shrinkage occurred convergent to the center. Invasive lesions were observed in only 2 cases, but they occupied less than 10% of the whole tumor area.

In the 9 cases that were observed by TS-CT over a period, a longer VDT was noted; the mean VDT was 1165 days. Lillington[14] reported that the VDT of benign tumors was more than 450 days and the VDT of malignant tumors was less than 450 days. Aoki et al[15] reported that the VDT of adenocarcinomas was 124 to 1486 days. The BLA-type adenocarcinomas have slow growth rates like BAC.[15] Adenocarcinomas with BLA have a slow growth rate because they are pathologically well differentiated, which include areas of collapse within the tumor that occur while a tumor is expanding. The expanding tumor areas and the shrinkage of the actual tumor (due to simultaneous collapse) occur at the same time while the tumor area expands. Adenocarcinomas with BLA are often diagnosed as old inflammatory lesions because of their specific TS-CT findings and slow growth.

Postoperative recurrence of BLA-type lung adenocarcinoma was noted in just 1 case. The 5-year survival rate is, at this time, 100%. It was reported that the smaller the invasive area, the better the prognosis. Hence, we can conclude that BLA-type adenocarcinomas have a good prognosis.[16] Usuda et al[7] reported that growth rates are important considerations when determining the prognosis. They wrote that the slow-growth group had a VDT longer than 113.3 days, whereas the rapid-growth group had a VDT of shorter than 113 days. The prognosis for the slow-growth group is better than that of the rapid-growth group. According to the pathological findings and the shorter VDT, we can conclude that BLA-type adenocarcinomas have a good prognosis.

In most of our 26 cases, it was difficult to accurately diagnose adenocarcinomas by a bronchoscope alone before the operation. In 21 cases, bronchoscopic studies were performed. Seven cases were diagnosed as adenocarcinoma by cytologic examination. Fourteen cases from classes 1 to 3 on cytologic examination could not be accurately diagnosed as carcinomas. We found that it is difficult to diagnose a malignancy because most parts of a tumor consist of collapsed areas and there are few cancer cells within the collapse area. Positron emission tomography was performed in 7 cases. Their maximum standardized uptake value (data not shown) was between 0.64 and 2.01. These are low marks, although the sizes were over 3 cm.[17] Consequently, we concluded that the unique TS-CT findings are the most important diagnostic consideration before an operation. Seventeen cases could not be pathologically diagnosed before the operation. Four of the 17 cases were diagnosed as lung cancer because the tumors grew during the observation periods. Nine of the 17 cases were diagnosed as BLA-type adenocarcinoma because they had all the BLA characteristic TS-CT findings. The patients underwent pulmonary lobectomy. In 2 cases, it was very difficult to diagnose a malignancy, so a pathological diagnosis was necessary during the operation.

In this study, *EGFR* mutations were detected in 65% of the BLA-type adenocarcinomas. We have reported that *EGFR* mutations were detected in approximately 60% of surgically resected lung adenocarcinomas in our hospital.[9] Thus, the *EGFR* mutational frequency in the present study is compatible with our previous report. It is of note that lung adenocarcinomas with nonmucinous BAC components have *EGFR* mutations more frequently than those without the components, and all of the 26 examined tumors with BLA had nonmucinous BAC components. On the other hand, approximately 70% of the *EGFR* mutations in BLA adenocarcinomas were clustered in the L858R point mutation. This L858R mutational frequency is inconsistent with well-known facts that L858R point mutations in exon 21 and deletion mutations in exon 19 are detected in almost equal frequency in lung adenocarcinomas.[18,19] This high frequency of the L858R point mutation may be a characteristic of BLA adenocarcinomas and might be related to biological characteristics of slow-growing tumors.

CONCLUSIONS

We also recommend that adenocarcinomas with BLA be more carefully observed because they can so easily be misdiagnosed as old inflammation. It is important to pay special attention to the unique characteristics of lung cancers. The TS-CT findings are a very important diagnostic tool, and we need to learn to evaluate these findings more accurately. We must confirm and correctly evaluate peripheral GGO areas and/or other characteristic findings.

TABLE 5. *EGFR* Mutation Analysis

EGFR mutations, No. cases	17/26
Details of mutation-positive cases, No. cases	
Male/female	4/13
L858R mutation	12/17
Exon 19 deletion	5/17
No mutations, No. cases	9/26
K-*ras* mutations, No. cases	0/26

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Original Article

Histological progression of small intrapulmonary metastatic tumor from primary lung adenocarcinoma

Keiju Aokage,[1,2] Genichiro Ishii,[1] Junji Yoshida,[2] Tomoyuki Hishida,[2] Mitsuyo Nishimura,[2] Kanji Nagai[2] and Atsushi Ochiai[1]

[1]*Pathology Division, Research Center for Innovative Oncology and* [2]*Division of Thoracic Surgery, National Cancer Center Hospital East, Kashiwa, Chiba, Japan*

The histopathology of small metastases is thought to reflect the early metastatic process. To clarify the morphological features of early metastatic tumor progression, we analyzed the histological heterogeneity of many small intrapulmonary metastases. Histological typing based on the World Health Organization classification (bronchioloalveolar carcinoma, acinar, papillary, and solid subtype) was used to evaluate 234 metastases from the primary lung adenocarcinomas of 139 patients. The predominant subtype of metastasis 3 mm or less in diameter was bronchioloalveolar carcinoma when the primary lesion was diagnosed as predominant bronchioloalveolar carcinoma, acinar, and papillary subtype. When the histology of the primary tumor was predominantly a solid subtype, the predominant subtype of metastatic tumor was also a solid subtype. However, analysis of metastases that were more than 3 mm showed that the predominant subtype of the metastasis reflected the predominant subtype of the primary tumor. Furthermore, we evaluated the number of subtypes in primary and metastatic tumors. As the metastasis grew larger, the number of subtypes in the metastatic lesion increased and came close to the number composed in the primary lesion. These findings suggest that implanted cancer cells display lepidic growth in the early metastatic phase and recapitulate the morphological heterogeneity of the original tumor as the metastasis enlarges.

Key words: heterogeneity, intrapulmonary metastasis, lung adenocarcinoma

Adenocarcinoma is the most common histological type of primary non-small cell lung cancer (NSCLC) and the tumors are often histologically heterogeneous. Lung cancer heterogeneity, especially of adenocarcinoma, has been recognized since the 1950s.[1,2] This characteristic has provided various discussions for many pathologists over the past several decades. Although, the World Health Organization (WHO) classification of lung adenocarcinoma has changed several times, in 2004 the WHO classified adenocarcinoma of the lung into five distinct subtypes: bronchioloalveolar carcinoma (BAC); acinar adenocarcinoma; papillary adenocarcinoma; solid adenocarcinoma with mucin production; and mixed subtype.[3,4] Since most adenocarcinomas are histologically heterogeneous, consisting of two or more histological subtypes, the majority of adenocarcinomas of the lung are classified as the mixed subtype.[4] The histological heterogeneity is an important feature of the pathology of adenocarcinoma of the lung, which consists of a mixture of histological types that represent derivation of lung cancer from pluripotent stem cells.

Metastasis, the spread of cancer cells from the primary lesion to form distant lesions, leads to the death of almost all cancer patients. Metastasis is a complex, multistep process that ultimately results in the formation of a mature tumor. Many investigators have elucidated some of the molecular mechanisms that underlie many of events involved in tumor metastasis, and three possible mechanisms of intrapulmonary metastasis have been postulated and described: a mechanism via the lymphatic vessel route; a mechanism via the blood vessel route; and a mechanism via the airway

Correspondence: Genichiro Ishii, MD, PhD or Atsushi Ochiai, MD, PhD, Pathology Division, Research Center for Innovative Oncology, National Cancer Center Hospital East, 6-5-1 Kashiwanoha, Kashiwa-city, Chiba-prefecture, 277-8577, Japan. Email: gishii@east.ncc.go.jp (G. Ishii), aochiai@east.ncc.go.jp (A. Ochiai)

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route.[5] Metastatic tumor cells generally separate from the primary tumor and attach to the vascular or lymphatic endothelium. A few cells extravasate and proliferate to form cell clusters. They recruit inflammatory cells to their cancer associated stroma and promote angiogenesis interacting with their stromal cells. Recent evidence suggests that the ability of cancer cells to metastasize reflects an inherent property of some cancer stem cells.[6–9] However, questions remain about the early metastatic process proliferating as colonies and forming a tumor within parenchyma.[10]

The lung is the most common site of metastases of primary NSCLC,[11] and small occult intrapulmonary metastatic tumors (micrometastases of primary lung cancer) are often detected in surgical specimens under the light microscope. The histopathology of small metastatic tumors is thought to reflect the early metastatic process of adenocarcinoma of the lung. The aim of this study was to elucidate the morphological characteristics and tumor progression of metastatic tumor from lung adenocarcinoma with especially in relation to size and histological heterogeneity.

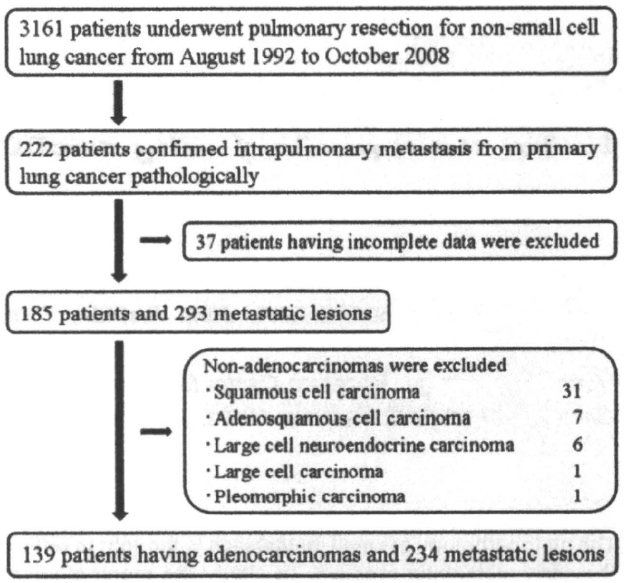


Figure 1 Consort diagram of this study. One hundred and thirty-nine patients with adenocarcinoma and a total of 234 intrapulmonary metastases were analyzed as an available cohort.

MATERIALS AND METHODS

Patient selection

Intrapulmonary metastases from primary lung cancer was detected in the resected specimens of 222 of the 3161 consecutive NSCLC patients who underwent surgical resection at the National Cancer Center Hospital East, Chiba, Japan, between July 1992 and October 2008. After excluding the 37 patients with incomplete data and the 46 patients diagnosed with non-adenocarcinoma, the remaining 139 patients diagnosed with adenocarcinoma and their 234 metastatic lesions in the lung were evaluated (Fig. 1). The median size of the primary tumors was 37 mm in diameter. Forty primary tumors were located in the right upper lobe (28.8%), 11 tumors in the right middle lobe (7.9%), 38 tumors in the right lower lobe (27.3%), and 19 tumors in the left lower lobe (13.7%).

In November 2008 the institutional review board approved the data collection and analyses and waived the need to obtain informed consent from each patient.

Histological studies

The surgical specimens were fixed in formalin or 100% methanol. These were sliced at the maximum diameter of the primary tumors and the subdivided sections of the cut surface were embedded in paraffin. Additionally, we identified each peripheral subsegmental bronchus and routinely cut slices of the divided sections involving the subsegmental bronchus even if there were no lesions. These sections were also embedded in paraffin. The median number of tissue blocks per resected specimen was 23. Serial 4 μm sections were stained with HE, by the Alcian blue-periodic acid-Schiff (AB-PAS) method for cytoplasmic mucin production, and by the Elastica van Gieson (EVG) or Victoria-blue van Gieson (VVG) stain method for elastic fibers. The primary tumors were diagnosed histologically based on the WHO classification of cell types and divided into five subtypes: BAC subtype (non-mucinous BAC or mucinous BAC); acinar subtype; papillary subtype; solid adenocarcinoma with mucin production subtype; and a mixed subtype.[3] We also identified the predominant subtype in the mixed subtype lesions. Lymphatic permeation was evaluated on Hematoxylin-Eosin (HE) stained sections and D2-40 stained sections, and vascular invasion was evaluated by examining EVG and VVG stained sections. The pulmonary metastasis (PM) category was divided into PM1, for a metastatic lesion in the same lobe as the primary lesion, and PM2, for a metastatic lesion in a different lobe from the primary lesion.

Histological subtyping of the metastatic tumors was performed according to the same classification used for the primary tumor, and the predominant subtype was also identified. The extent of fibrosis in the metastatic foci was evaluated as none/mild and moderate/severe. We also examined the section for the state of alveolar septal destruction (assessed by VVG staining) and the extent of lymphocyte infiltration (none or mild and moderate or severe). These morphological features of the metastatic tumor were statistically compared in the two groups formed by dichotomizing by the median diameter of the metastases of 3 mm.

Histological criteria for the diagnosis of metastatic tumor

All histological materials included in this series were initially assessed for diagnosis by pathologists. The materials were subsequently reviewed by two pathologists (K. A. and G. I.) to confirm the presence of pulmonary metastasis and assess the histopathological features of both the primary and metastatic tumors. An intrapulmonary metastasis was diagnosed according to the following definitions: (i) primary tumor having vascular invasion and/or lymphatic permeation, a secondary tumor having the same cytological feature including nuclear atypia as the primary tumor, and the secondary tumor cells showing severe nuclear atypia even at the peripheral region; or (ii) the existence of many scattered floating cancer cells in alveolar space and small satellite lesions surrounding the primary tumor and a secondary tumor having the same cytological feature including nuclear atypia as the primary tumor, as well as the secondary tumor cells showing severe nuclear atypia even at the peripheral region. These criteria were obtained by partially modifying the criteria established by Martini and Melamed.[12] To prove the validity of our differential diagnoses we analyzed the survival of this population using the Kaplan-Meier Method. Pathological staging was based on the seventh TNM Classification of Malignant Tumors.[13] The 5 year survival rates of pathological stage IIB, IIIA, and IIIB/IV of this study were 40.8, 25.6, and 8.5%, respectively. These results were similar to previous international data, supporting the validity of our diagnosis.[14,15]

Histological subtype heterogeneity index

The histological subtype heterogeneity index of a metastatic tumor was defined as the ratio of the number of subtypes composing the metastatic tumor to the number of subtypes composing the primary tumor and expressed as a decimal; if the primary tumor was composed of a BAC, acinar, and papillary component (three subtypes) and the metastatic tumor were composed of a BAC and papillary component (two subtypes), the histological subtype heterogeneity index would be 2/3 (0.67). The possible index range is from 0.25 (1/4) to 1.00 (1/1, 2/2, 3/3, and 4/4). The histological subtype heterogeneity indexes of the two groups formed by dichotomizing the metastases by their median size of 3 mm were statistically compared in each denominator of the number of subtypes in the primary tumor from 1 to 4. There was no intrapulmonary metastatic tumor whose number of subtypes exceeded the number of subtypes composed in the primary tumor in this study. Therefore, the histological subtype heterogeneity indexes never exceeded 1.00.

Statistical analysis

Differences in patient characteristics between the two groups were tested for significance with Pearson's chi-squared test. The unpaired *t*-test was performed to calculate the statistical significance of differences. All *P* values reported are two-sided, and the significance level was set at less than 0.05. The analyses were performed with the SPSS 11.0 statistical software program (Dr SPSS II for Windows, standard version 11.0, SPSS Inc., Chicago, IL, USA) and GraphPad Prism statistical software program (Prism for Windows, Version 5.02, GraphPad Software, Inc., La Jolla, CA, USA).

RESULTS

Clinicopathological findings

The clinicopathological data of the 139 patients diagnosed with adenocarcinoma are summarized in Table 1. Intrapulmonary metastases were detected occasionally in the resected specimen of 121 patients (87.1%). Eighteen patients (12.9%) were diagnosed as having intrapulmonary metastasis preoperatively. Forty-seven patients (33.8%), 78 patients (56.1%), 9 patients (6.5%), and 5 patients (3.6%) were pathologically diagnosed as stage IIB, IIIA, IIIB, and IV, respectively. Their survival curves are depicted in Fig. 2. The 5 year survival rates of pathological stage IIB, IIIA, and IIIB/IV in this study were 40.8, 25.6, and 8.5%, respectively. The histological subtypes of the primary tumor in the 139 lung adenocarcinoma patients were: mixed subtype adenocarcinoma in 134 patients (96.4%); pure BAC subtype in 0 patients (0%); acinar subtype in 0 patients (0%); papillary subtype in 1 patient (0.7%); and the solid adenocarcinoma with mucin production subtype in 4 patients (2.9%) (Table 1).

The histological subtypes of the 234 metastatic lesions detected in the 139 patients were: mixed subtype adenocarcinoma in 166 metastases (70.9%); pure BAC subtype in 33 metastases (14.1%); acinar subtype in 2 (0.9%); and papillary subtype in 28 (12.0%). Their sizes are indicated in a histogram (Fig. 3). The median diameter of the lesions was 3 mm.

Comparison between the predominant histological subtypes in the primary tumors and the metastatic tumors

Of the 139 primary tumors diagnosed, 20 tumors (14.4%) contained four subtypes (BAC, papillary, acinar, and solid), 54 tumors (38.8%) contained three subtypes, 60 tumors (43.2%) contained two subtypes, and five tumors (3.6%) were composed of a single subtype. Of the 234 metastatic

Table 1 Characteristics of patients with intrapulmonary metastasis from primary lung adenocarcinoma

Factors	n = 139 (%)
Age (years)†	
<66	65 (46.8)
≥66	74 (53.2)
Sex	
Male	78 (56.1)
Female	61 (43.9)
Smoking history	
Never a smoker	59 (42.4)
Current / previous smoker	80 (57.6)
CEA level (ng/mL)	
<5.0	81 (58.3)
≥5.0	58 (41.7)
Preoperative pulmonary metastasis	
PM0	121 (87.1)
PM1	13 (9.4)
PM2	5 (3.6)
Histological subtype in primary lesion	
Adenocarcinoma, mixed subtype	134 (96.4)
BAC	
Mucinous BAC	0 (0)
Non-mucinous BAC	0 (0)
Acinar adenocarcinoma	0 (0)
Papillary adenocarcinoma	1 (0.7)
Solid adenocarcinoma with mucin production	4 (2.9)
Pathological n classification	
pN0	51 (36.7)
pN1	28 (20.1)
pN2	57 (41.0)
pN3	3 (2.2)
Pathological stage (UICC7)	
Stage IIB	47 (33.8)
Stage IIIA	78 (56.1)
Stage IIIB	9 (6.5)
Stage IV	5 (3.6)
Pathological pulmonary metastasis	
PM1	128 (92.1)
PM2	11 (7.9)
Lymphatic permeation	
Present	90 (64.7)
Absent	49 (35.3)
Vascular invasion	
Present	96 (69.0)
Absent	43 (30.9)
Number of pulmonary metastatic tumors	
1	79 (56.8)
2	22 (15.8)
3	20 (14.4)
More than 5	18 (12.9)

†Age was dichotomized by the median age of 66 year-old.

BAC, bronchioloalveolar carcinoma; CEA, carcinoembryonic antigen; PM0, no metastatic lesion in the lung clinically; PM1, metastatic lesion in the same lobe as the primary lesion; PM2, a metastatic lesion in a different lobe from the primary lesion; UICC, International Union Against Cancer.

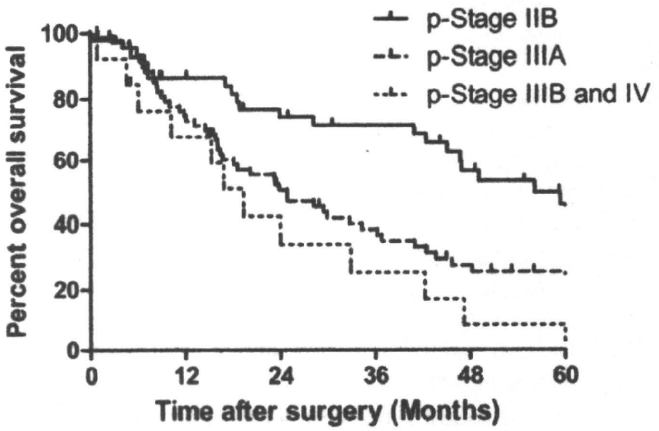


Figure 2 Overall survival curves of patients with intrapulmonary metastasis according to pathological stage (stage IIB, stage IIIA, stage IIIB and IV).

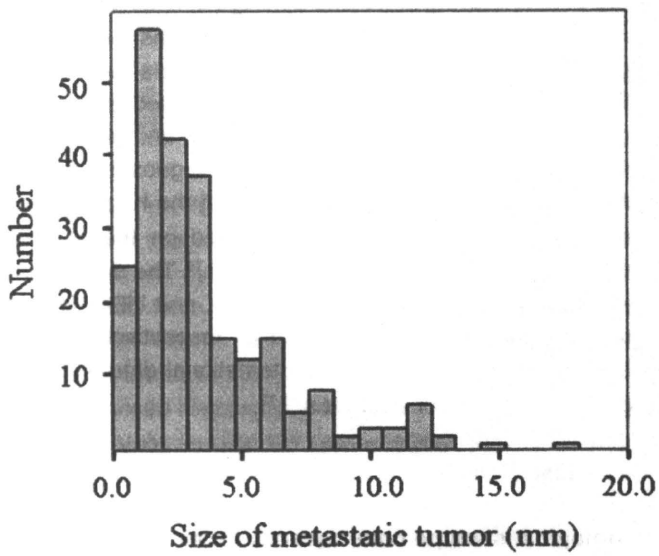


Figure 3 Size histogram of the intrapulmonary metastatic tumors from primary lung cancers. Their median diameter was 3 mm.

tumors, one tumor (0.4%) contained four subtypes, 15 tumors (6.4%) contained three subtypes, 150 tumors (64.1%) contained two subtypes, and 68 tumors (29.1%) were composed of single subtype. We compared the predominant histological subtype of the primary tumors and metastatic tumors in each of the 228 cases in which the primary tumor was a mixed subtype adenocarcinoma (Fig. 4a,b). Focusing on the examination of the metastatic tumor whose diameter was 3 mm or less, we found that the predominant subtype in 76.9% of the metastastic tumors which revealed the BAC predominant in the primary tumor was BAC subtype (Fig. 5a,b). The predominant subtype in 78.6% and 41.3% of the metastatic tumors which showed the predominant acinar subtype and the predominant papillary subtype in the primary tumor, respectively, was also BAC subtype (Fig. 5d,e,g,h).

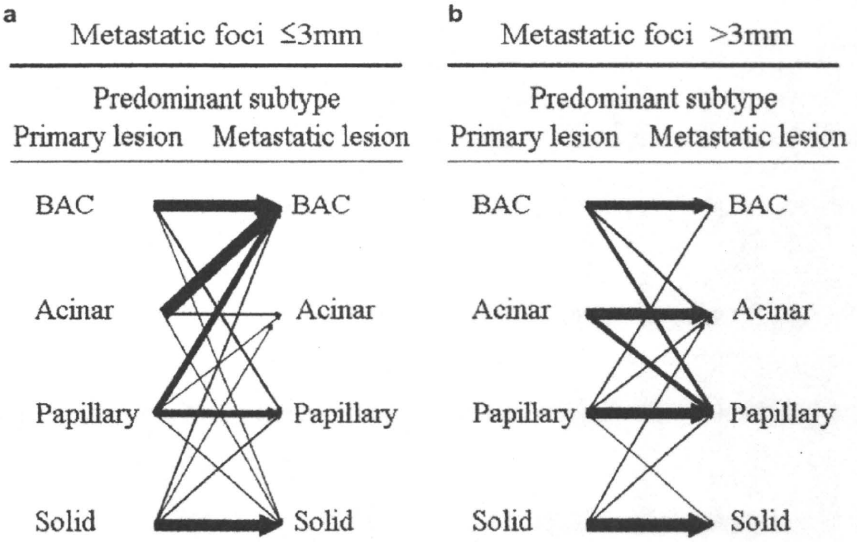


Figure 4 Correlation between predominant histological subtype in the primary lesion and the metastatic lesions according to the size of the metastatic tumors; (**a**) 3 mm or less; (**b**) more than 3 mm. The thickness of black arrows reveals the percentage of cases. BAC, bronchioloalveolar carcinoma.

When the histology of the primary tumor was predominantly the solid subtype, the most predominant histological subtype of the metastatic tumors was the solid subtype (70.6%; Fig. 5j,k), the predominant subtype of 11.8% of them was the BAC subtype.

By contrast, the predominant histological subtypes of the metastatic tumors that were more than 3 mm in diameter were basically the same as the predominant subtypes of the primary tumors (BAC/BAC; 58.3%, acinar/acinar; 66.7%, papillary/papillary; 70.0%, solid/solid; 73.3%; Fig. 4b).

Histological heterogeneity index

To investigate how the histological heterogeneity in the primary tumor is maintained in the metastatic tumors, we compared the number of the histological subtypes composing the primary tumors and metastatic tumors in each case and calculated the histological heterogeneity index (see materials and method). The proportion of cases with an index of 1.00, which means that the number of histological subtypes in the primary lesion and the metastatic lesion was the same, was 36.5% in the group with metastatic tumors 3 mm or less and 53.6% in the group with metastases more than 3 mm in diameter. The histological heterogeneity index of the group indicating one subtype 1.00 in both groups, indicating that all metastatic tumors whose primary lesions composed of a single histological subtype also showed single histological subtype (Fig. 6a). Figure. 6b,c,d show comparisons between the two size groups where the number of histological subtypes composing the primary tumor was 2, 3, and 4. The groups indicating 2 subtypes had no statistical difference. However, the histological heterogeneity index of the groups indicating 3 and 4 had significant difference (Fig. 6c,d, $P = 0.003$ and $P = 0.024$). The mean ± standard error (SE) of this index in the group of metastatic tumors whose diameter was 3 mm or less and the group of metastatic tumors whose diameter was more than 3 mm was 0.57 ± 0.03 and 0.72 ± 0.04, respectively (Fig. 6c), and 0.44 ± 0.03 and 0.59 ± 0.08, respectively (Fig. 6d). These findings imply that as metastatic lesions grow larger their cancer cells display diverse growth patterns resulting in histological heterogeneity as seen in the primary lesions.

We also examined the histological subtype heterogeneity of the primary subcentimeter adenocarcinoma and the metastatic adenocarcinoma matched in size (6–10 mm). The number of histological subtypes in the metastatic tumor (mean ± SE; 1.94 ± 0.10, n = 33) was significantly higher than that in the primary subcentimeter adenocarcinoma (1.58 ± 0.11, n = 40) (Data not shown; $P = 0.01$).

Comparison of the pathological characteristics of the metastatic lesions according to size

Table 2 shows the differences between the morphological findings in the metastatic tumors according to their size. The metastatic tumors 3 mm or less in diameter were composed of mainly predominant BAC subtype. Less destruction of alveolar septa and less intense stromal reactions including lymphocyte infiltration and fibrosis were observed in the group of metastasis 3 mm or less in diameter, and the differences were statistically significant.

DISCUSSION

Metastasis is the final step in neoplastic progression, and many investigators have made great efforts to elucidate its

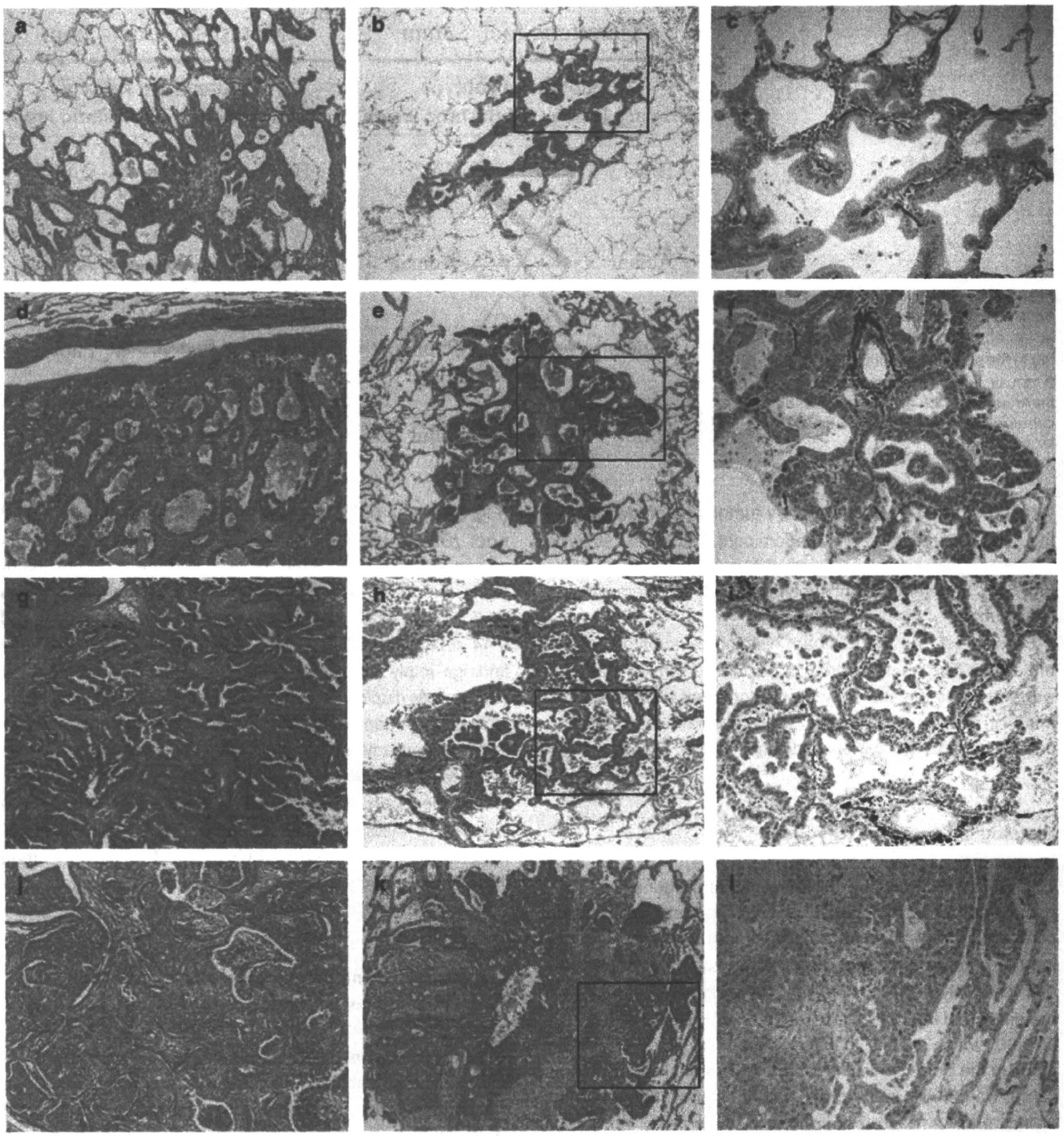
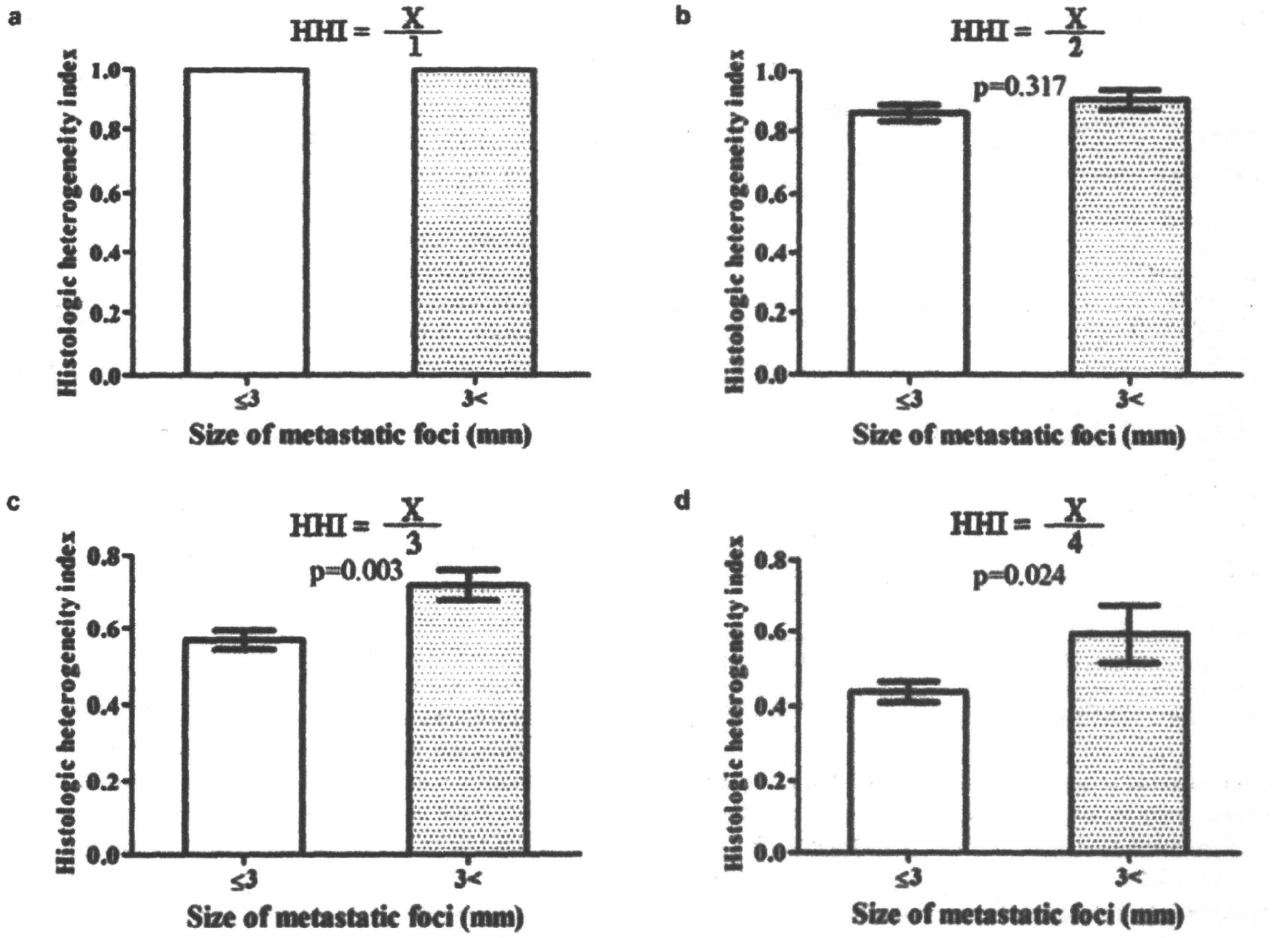


Figure 5 Histopathological features of the primary tumors and metastatic tumors. (**a**) The primary tumor whose predominant subtype is the bronchioloalveolar carcinoma (BAC) subtype (HE stain). (**b**) This metastatic tumor from the primary lesion in Figure 5a was mainly composed of a BAC component. (**c**) Higher magnification view of the tumor in Figure 5b. The pulmonary parenchyma is preserved (Victoria-blue van Gieson (VVG) stain). (**d**) The primary tumor whose predominant subtype is the acinar adenocarcinoma subtype exhibiting endobronchial growth (HE stain). (**e**) This metastatic tumor from the primary lesion in Figure 5d was mainly composed of a BAC component (HE stain). (**f**) Higher magnification view of the metastatic tumor in Figure 5e. The pulmonary parenchyma is preserved (VVG stain). (**g**) The primary tumor whose predominant subtype is the papillary adenocarcinoma subtype (HE stain). (**h**) This metastatic tumor from the primary lesion in Figure 5g is predominantly composed of the BAC subtype and has a micropapillary component (HE stain). (**i**) Higher magnification view of the metastatic tumor in Figure 5h. The pulmonary parenchyma is preserved (VVG stain). (**j**) The primary tumor whose predominant component is the solid adenocarcinoma subtype (HE stain). (**k**) This metastatic tumor from the primary lesion in Figure 5j is mainly composed of a solid component (HE stain). (**l**) Higher magnification view of the metastatic tumor in Figure 5k. The pulmonary parenchyma had been destroyed (VVG stain).



$$\text{Histologic heterogeneity index (HHI)} = \frac{\text{The number of subtype composed of the } \textit{metastatic tumor}}{\text{The number of subtype composed of the } \textit{primary tumor}}$$

Figure 6 The histological subtype heterogeneity index of the metastatic tumors was calculated by dividing the number of subtypes composing the metastatic tumor by the number of subtypes in the primary tumor. The metastatic tumors were dichotomized by their median diameter of 3 mm. (**a**) The comparison in the population indicating a denominator of 1 is demonstrated. The mean of this index in both groups was 1.00. (**b**) Comparison in the lesion with a denominator of 2 did not reveal a difference ($P = 0.317$). (**c**) The comparison in the lesion with 3 as the denominator revealed a difference, that was statistically significant ($P = 0.003$). (**d**) The comparison in the lesion with 4 as the denominator revealed a difference, that was statistically significant ($P = 0.024$).

mechanisms and to control it. Metastasis is a complex, multistep process and it is not easy to grasp the entire metastatic process. Although the morphology of micrometastases of lung cancer in regional lymph nodes has been described, the architecture of the lungs and lymph nodes are clearly different, and that would influence the growth pattern of the cancer cells. Since the aim of this study was to analyze the morphological characteristics of metastatic tumors that grew at a site with the same architecture as the organ that was the site of the primary tumor, we investigated small intrapulmonary metastatic lesions. This is the first report of a study that analyzed a large number of small intrapulmonary metastases in detail by applying the histological classification of primary adenocarcinoma of the lung to metastatic tumors. The proportion of metastatic tumors whose number of histological subtypes was the same as the number in the primary lesion was 36.5%, even when the size of the metastases was 3 mm or less. As a result, we were able to conclude that most metastatic tumors exhibit histological heterogeneity even in the early phase of the metastatic process. Moreover, the larger the metastatic tumors were, the higher heterogeneity the metastatic tumors showed and these eventually displayed similar histological components to primary adenocarcinoma of the lung. There are several possible explanations for these results. Cancer stem cells (CSC) have been found to be important in tumor initiation, and their importance has

Table 2 Relationship between size and pathological factors in metastatic tumors (n = 234)

Factors	Size of metastatic tumor† (mm) ≤3	>3	*P*-value‡
Predominant subtype			
BAC	76	12	
Non-BAC	85	61	<0.001
Acinar	9	11	
Non-acinar	152	62	0.016
Papillary	52	34	
Non-papillary	109	39	0.036
Solid	24	16	
Non-solid	137	57	0.187
Destruction of alveolar septa			
Absent	106	22	
Present	55	51	<0.001
Lymphocyte infiltration			
None or Mild	112	62	
Moderate or Severe	49	11	0.013
Fibrosis			
None or Mild	140	37	
Moderate or Severe	21	36	<0.001

†Size of metastatic tumor was dichotomized by median size of 3 mm.
‡Pearson's chi-squared test.
BAC, bronchioloalveolar carcinoma.

been reported in a variety of solid tumors. The role of CSC in cancer progression, particularly with respect to metastasis, also has been suggested.[6,7,16] Pluripotent stem cells from the primary lesion may play an important role in the formation of metastatic foci; therefore these also display histological heterogeneity in the metastatic lesion, as many investigators have suggested in the past. Britta *et al.* suggested that an inherent feature of most breast cancers is that they are sustained throughout the metastatic process Britta *et al.* showed that distant metastases display both the same molecular breast cancer subtype as well as the 70-gene prognosis signature as their primary tumors.[17] Taking their suggestion into consideration, our results showing that as the metastatic tumor becomes larger it increasingly resembles the primary lesion morphologically are acceptable.

However, regarding small metastatic tumors 3 mm or less in diameter, we found a distinctive growth pattern. It is very interesting that the predominant subtype in 47.2% (76/161) of all metastatic tumors 3 mm or less in diameter was BAC subtype. 76.9% of predominant BAC subtype in the primary tumor also resulted in predominant BAC subtype in their metastatic tumor; 78.6% of predominant acinar subtype and 41.7% of predominant papillary subtype in the primary tumor also predominant BAC subtype in the metastatic tumor. This may mean that cancer cells that migrate from primary tumors whose predominant subtype is BAC subtype, acinar subtype, and papillary subtype proliferate along the alveolar septa, thereby giving rise to a lepidic growth pattern in their early metastatic lesions. Generally, the cancer cells that metastasized may proliferate using the pre-existing structure in the target organ in the early phase of metastasis. However, these cells, having pluripotency, generate metastatic foci that recapitulate the morphological heterogeneity of the original tumor as the metastatic tumor grows. On the other hand, both the small and large metastatic tumors of predominant solid adenocarcinoma subtype were the same subtype as the primary tumor. Tumor cells that have metastasized from predominant solid adenocarcinomas may tend to form a solid nest that destroys the alveolar septa even in the early phase of metastasis, so they are considered to display low affinity against pre-existing alveolar septal structure. This phenomenon implies that they belong to another biological spectrum different to the other three subtypes. Actually, some investigators have shown that solid adenocarcinomas express genetic features that are different to those of other subtypes, including a lack of epidermal growth factor receptor mutations, loss of WW domain-containing oxidoreductase, a high frequency of loss of heterozygosity, and a high grade of chromosome instability.[4,18–22]

Recently, one of the histological subtypes of the micropapillary subtype was suggested to have a correlation with the patient's prognosis and metastasis to the lung. In the current study, among the 139 primary lung adenocarcinoma patient with pulmonary metastasis, 73 patients had a micropapillary component in the adenocarcinoma (52.5%). Forty-two of 161 lesions (26.1%) of the metastatic tumors whose diameters were 3 mm or less included a micropapillary component, whereas the 43.8% (32 of 73 cases) of metastatic tumors whose diameters were more than 3 mm included a micropapillary component. As the metastatic tumors grew in size, the percentage of metastatic tumors with a micropapillary component increased.

Differential diagnosis between intrapulmonary metastasis and multiple primary tumors is always a topic of debate and sometimes difficult to clarify.[12,23] In this study, we diagnosed the difference by putting emphasis on histological and cytological morphology. Furthermore the finding that the atypia of tumor cells at the peripheral area is fairly mild, which is often observed in the peripheral BAC areas of the primary lung cancer, was not observed in any of the metastatic tumors. The 5 year survival rates of pathological stage IIB, IIIA, and IIIB/IV in this study were 40.8, 25.6, and 8.5%, respectively. These results were similar to the previous international data, supporting the validity of our diagnosis.[14,15] Girard *et al.* described the importance of comprehensive histological assessment for a differential diagnosis including histological subtyping, cytological features, and stromal characteristics in the case of mature metastasis.[24,25] However, they analyzed tumor specimens by using array comparative genomic hybridization or mutational profiling of select genes. Genomic examination like this is necessary in future analysis.

The number of histological subtypes in the metastatic tumor was significantly higher than that in the primary subcentimeter adenocarcinoma. That indicates the difference of growth patterns between the primary tumor and the metastatic tumor in tumor progression.

In conclusion, the results of our study showed that most metastatic tumors from primary adenocarcinoma other than solid adenocarcinomas exhibited a predominant BAC subtype in the early phase and recapitulated the morphological heterogeneity of the original tumor as the tumor grew. This phenomenon reflects the process of tumor progression in intrapulmonary metastasis by primary adenocarcinoma of the lung. These small lung metastases from primary adenocarcinoma may provide as optimal model for elucidating the molecular mechanisms of early metastasis.

ACKNOWLEDGMENTS

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Long-Term Outcome and Late Recurrence in Patients with Completely Resected Stage IA Non-small Cell Lung Cancer

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

Background: The purpose of this study was to quantify the risk of late recurrence in patients with stage IA non-small cell lung cancer (NSCLC) who remained recurrence-free for more than 5 years after resection.

Methods: Between August 1992 and December 2002, a total of 519 patients with stage IA NSCLC underwent complete resection at our institution. Recurrence-free probability was measured from the benchmark of 5 years after primary tumor resection to the date of first recurrence or last follow-up using the Kaplan-Meier method.

Results: Of a total of 519 patients, 434 remained recurrence-free for 5 years. Among these, 21 (4.8%) developed late recurrence more than 5 years after resection. Recurrence-free interval ranged from 1 to 53 months, and the median recurrence-free interval was 14 months from the benchmark of 5 years after primary tumor resection. The 5-year recurrence-free probability from the benchmark was 93%. Multivariate Cox analysis demonstrated that vascular invasion significantly influenced late recurrence ($p = 0.038$). The 5-year recurrence-free probability from the benchmark was 84% for patients with vascular invasion and 95% for patients without vascular invasion ($p < 0.001$).

Conclusions: Patients with stage IA NSCLC with vascular invasion harbor a significant risk of late recurrence more than 5 years after complete resection. In patients with stage IA NSCLC with vascular invasion, 5 years without recurrence is not sufficient to conclude that NSCLC is cured. In contrast, patients without vascular invasion may be declared to be cured at 5 years after resection if they are recurrence-free.

Key Words: Non-small cell lung cancer, Late recurrence, Stage IA, Thoracic surgery.

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In Japan, the recent increase in the frequency of detecting stage IA non-small cell lung cancer (NSCLC) can be attributed to a nationwide mass screening system.[1] For these early-stage NSCLC patients, complete surgical resection provides the best possibility of cure.

The reported 5-year survival rates of patients with resected stage IA NSCLC ranged from 80 to 93%.[2–6] Although many researchers have studied postoperative recurrence and its risk factors in patients with stage IA NSCLC within the first 5 years after resection,[1] information about the survival of such patients beyond 5 years is limited.[4,7]

This study reviewed a large series of consecutive patients with stage IA NSCLC who had been operated on in a single institution. Because of its long follow-up period, this study was able to focus on long-term outcome and late recurrence. The purposes of this study were to quantify the risk of late recurrence in patients with stage IA NSCLC who survived more than 5 years after resection and to suggest long-term follow-up strategy.

PATIENTS AND METHODS

A total of 541 patients with pathologic stage IA NSCLC, who underwent complete resection between August 1992 and December 2002, were identified in our departmental database. Complete resection was defined as cancer-free surgical margins both grossly and histologically. Twenty-two patients were excluded because of (1) preoperative chemotherapy or radiation therapy, or both ($n = 6$), and (2) low-grade pulmonary malignancies including carcinoid tumor, mucoepidermoid carcinoma, or adenoid cystic carcinoma ($n = 16$). The remaining 519 patients were the study subjects.

Pathologic Evaluations

Histologic type was determined according to the World Health Organization classification,[8] and histologic grade was diagnosed and categorized into well, moderately, and poorly differentiated carcinomas according to the degree of structural and cytologic atypia. Differentiation in squamous cell carcinoma was determined based on degree of keratinization, intercellular bridges, and squamous pearl formation. Poor differentiation in adenocarcinoma was defined as a solid pattern tumor without any clear gland formation. Blood and lymphatic vessels were identified by hematoxylin-eosin, elastin (Victoria blue-van Gieson), and D2-40 immunohistochemical stainings. Vascular invasion and lymphatic permeation were histologically diagnosed by identifying cancer cells within blood and lymphatic vessels, respectively. Disease stages were based on the tumor, node,

*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.

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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

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metastasis classification of the International Union Against Cancer, 7th edition.[9]

Patient Follow-Up

We examined patients at 3-month intervals for the first 2 years and typically at 6-month intervals thereafter on an outpatient basis. After 5 years from resection, we aimed at continuing follow-up annually for at least 5 more years. The follow-up evaluation included physical examination, chest radiography, and blood examination including pertinent tumor markers. Whenever any symptoms or signs of recurrence were detected, further evaluations were performed, including computed tomography scans of the chest and abdomen, brain magnetic resonance imaging, and bone scintigraphy. Since 2004, integrated positron emission tomography and computed tomography have also been performed when appropriate.

We diagnosed recurrence based on compatible physical examination and diagnostic imaging findings and confirmed histologically when clinically feasible. The date of recurrence was defined as the date of histologic proof or, in cases diagnosed based upon clinicoradiological findings, the date of identification by a physician.

In this study, we used the following criteria to define and differentiate metachronous primary lung cancers based on the modified criteria of Martini and Melamed[10]: tumors histologically different from the primary tumor and occurring at anatomically separate sites or tumors histologically the same as the primary tumor without systemic metastases or mediastinal spread, developing after a tumor-free interval of at least 2 years.

We classified recurrence into two categories: (1) locoregional disease was clinically or radiologically manifest in the ipsilateral mediastinum, pleural cavity, supraclavicular lymph nodes, or bronchial stump, and (2) distant disease was located in the contralateral lung or outside the hemithorax of the primary lesion.

Clinicopathologic Information

We reviewed the medical records of each patient for clinicopathologic information including age (dichotomized at the median age of 65 years), gender, smoking history (nonsmoker or ever-smoker), preoperative serum carcinoembryonic antigen level (cutoff at the normal upper limit of 5 ng/ml), extent of resection, maximum tumor dimension on the resected specimen ($\leq$2 cm or $>$2 cm), histology, histologic differentiation (well differentiated or moderately/poorly differentiated), lymphatic permeation (presence or absence), and vascular invasion (presence or absence).

Statistical Analysis

The length of overall survival was defined as the interval in months between the date of surgical resection and the date of either death or the last follow-up. Recurrence-free probability was calculated in months from the point of 5 years after resection to the date of the first recurrence or last follow-up. To calculate recurrence-free probability, patients who died without recurrence or who were known to be recurrence-free at the date of last contact were censored. For univariate analyses, all cumulative survival rates 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. Forward and backward stepwise procedures were used to determine independent predictors. Annual recurrence rates were determined by dividing the number of recurrences in a certain year (r) by the sum of the number of patients without recurrence for the entire year (p), the number of recurrent patients in the year (r), and a half of the number of patients without recurrence followed for less than the entire year (c) according to the following formula:

$$\text{Annual recurrence rate} = r/p + r + 0.5\ (c)^{[11-13]}$$

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 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 informed consent from each patient was waived by the institutional review board in August 2009.

RESULTS

Table 1 shows the clinicopathologic characteristics of 519 patients with stage IA NSCLC. The overall survival rates of the 519 patients with stage IA NSCLC at 5 and 10 years after resection were 88.0% and 74.4%, respectively. By 5 years after resection, 35 had died of disease and 32 had died of other causes. Eighteen were alive with disease. The remaining 434 patients were alive and recurrence-free for the first 5 years. The median follow-up period of these 5-year recurrence-free survivors was 44 months after the first 5 years (range, 1–86 months). Among the 434 patients, 21 (4.8%) developed recurrence. Recurrence was locoregional in 9 patients and distant in 12 patients (Table 2).

From the point of 5 years after primary tumor resection, the recurrence-free intervals ranged from 1 to 53 months, and the median recurrence-free interval was 14 months for the 5-year recurrence-free survivors. For these survivors, the recurrence-free probability was 93.3% at 5 years after the first 5 years. Table 3 lists the recurrence-free probabilities at 5 years after the 5-year benchmark date according to clinicopathologic features. On univariate analysis, ever-smoker, nonadenocarcinoma histology, poor/moderate histologic differentiation, and presence of vascular invasion were shown to be significant risk factors for late recurrence beyond 5 years. Recurrence-free probability was not statistically different between groups stratified by age, gender, preoperative serum carcinoembryonic antigen level, extent of resection, maximum tumor dimension on the resected specimen, histology, or lymphatic permeation.

On multivariate analysis with the Cox regression model, the presence of vascular invasion remained a statistically significant predictor for late recurrence more than 5 years after surgery (hazard ratio: 2.74, $p = 0.038$; Table 4). The recurrence-free probability at 5 years after the first 5 years for patients without vascular invasion was 95.2%, whereas that for patients with vascular invasion was 83.7% ($p < 0.001$; Figure 1).

TABLE 1. Clinicopathologic Characteristics of 519 Patients with Stage IA NSCLC

Characteristic	No. of Patients (%)
Overall number	519
Age (yr)	
<65	258 (49.7)
≥65	261 (50.3)
Gender	
Female	244 (47.0)
Male	275 (53.0)
Smoking habits	
Nonsmoker	236 (45.5)
Ever-smoker	283 (54.5)
CEA	
Within normal range	385 (74.2)
Elevated	132 (25.4)
Not measured	2
Extent of resection	
Lobectomy	467 (90.0)
Segmentectomy or wedge resection	51 (9.8)
Pneumonectomy	1
Tumor size (mm)	
≤20	217 (41.8)
>20	302 (58.2)
Histologic type	
Adenocarcinoma	416 (80.2)
Squamous cell carcinoma	80 (15.4)
Large cell carcinoma	14 (2.7)
Adenosquamous carcinoma	9 (1.7)
Histological differentiation	
Well differentiated	288 (55.5)
Moderately/poorly differentiated	231 (44.5)
Lymphatic permeation	
Absent	431 (83.0)
Present	88 (17.0)
Vascular invasion	
Absent	423 (81.5)
Present	96 (18.5)

Numbers in parentheses are percentages.
CEA, preoperative serum carcinoembryonic antigen level, normal upper limit at 5 ng/ml.

TABLE 2. Initial Site of Failure in 21 Patients with Late Recurrence

Initial Site	N
Distant metastasis	12
Multiple organ metastases	5
Single organ metastasis	7
Multiple lung metastases	2
Brain	2
Bone	1
Liver	1
Adrenal gland	1
Locoregional recurrence	9
Mediastinal lymph nodes	4
Pleural dissemination	2
Supraclavicular lymph nodes	2
Bronchial stump	1

The recurrence rate per year peaked in the second year after resection and gradually decreased thereafter but continued at a rate of 0.5 to 2% beyond 5 years until 10 years after resection (Figure 2). Figure 3 shows the recurrence rates per year stratified by vascular invasion status. The recurrence rate per year in patients without vascular invasion peaked in the third year after resection but decreased thereafter, whereas the recurrence rate per year in patients with vascular invasion peaked in the second year after resection and continued at a higher rate of 2 to 10% until 9 years from resection.

DISCUSSION

In patients with NSCLC, 5-year disease-free survival is currently the benchmark of cure. However, there are two issues with the follow-up of patient with NSCLC: When can cure be declared with confidence? For how long should follow-up examinations be continued? To examine these questions, we quantified the risk of late recurrence in patients with stage IA NSCLC who survived without recurrence for more than 5 years after resection. We were able to investigate long-term outcome in a large series of patients with stage IA NSCLC because of the long follow-up period (the median follow-up period after resection of 5-year recurrence-free survivors was 104 months).

Recurrence in NSCLC is most frequent during the first 2 years after resection, and more than 80% of recurrence occurs within this time.[4,14] In the current study, the recurrence rate per year in the entire cohort peaked in the second year after resection and gradually decreased thereafter (Figure 2).

Okada et al.[15] reported a 10-year survival rate of 91% among 421 5-year survivors of stage I to IIIB NSCLC, and Martini et al.[16] reported a 10-year survival rate of 92.4% among 686 5-year survivors of stage I to IIIA disease. Both studies concluded that late recurrence beyond 5 years was infrequent. They concluded that 5 years after resection is sufficient to declare that an NSCLC patient is cured because of the low recurrence incidence.

In this study involving only stage IA patients, the number of patients studied was 519, and 434 of them were recurrence-free at 5 years after resection. Recurrence developed in 4.8% of the 5-year recurrence-free survivors. Delayed recurrence was locoregional in 2.1% and distant in 2.8% of patients. These recurrence rates in patients with stage IA NSCLC were lower than those of the previous studies, including stage IB or higher patients.

Okada et al.[15] reported that disease stage, nodal status, gender, or histology did not affect long-term survival. By multivariate analyses, however, we identified one independently significant predictor of late recurrence in stage IA 5-year recurrence-free survivors: the presence of vascular invasion (hazard ratio: 2.74). The recurrence rate per year in patients without vascular invasion peaked at a rate of 2% in the third year after resection and gradually decreased thereafter. Although the recurrence rates per year in these patients did not reach zero until the 10th year after resection, they were less than 2% beyond 3 years after resection. In patients with vascular invasion, the

TABLE 3. Recurrence-Free Probability Beyond 5 yr After Complete Resection and Clinicopathologic Characteristics

Characteristic	No. of Patients (%)	No. of Recurrences (%)	Recurrence-Free Probabilities from 5-yr Benchmark at 5 yr (%)	Univariate *p* Value
Overall number	434	21 (4.8)	93.3	
Age (yr)				
<65	237 (54.6)	9 (3.8)	95.2	
≥65	197 (45.4)	12 (6.1)	90.7	0.211
Gender				
Female	222 (51.2)	7 (3.2)	95.4	
Male	212 (48.8)	14 (6.6)	91.2	0.072
Smoking habits				
Nonsmoker	217 (50.0)	5 (2.3)	97.3	
Ever-smoker	217 (50.0)	16 (7.4)	88.8	0.009[a]
CEA				
Within normal range	337 (77.6)	15 (4.5)	94.1	
Elevated	95 (21.9)	6 (6.3)	90.1	0.299
Not measured	2	0		
Extent of resection				
Lobectomy or pneumonectomy	395 (91.0)	19 (4.8)	93.5	
Segmentectomy or wedge resection	39 (9.0)	2 (5.1)	90.1	0.805
Tumor size (mm)				
≤20	174 (40.1)	7 (4.0)	94.1	
>20	260 (59.9)	14 (5.4)	92.9	0.400
Histologic type				
Nonsquamous cell carcinoma	376 (86.6)	14 (3.7)	95.1	
Squamous cell carcinoma	58 (13.4)	7 (12.1)	81.1	0.006[a]
Histological differentiation				
Well differentiated	263 (60.6)	8 (3.4)	96.0	
Moderately/poorly differentiated	171 (39.4)	13 (8.2)	88.2	0.009[a]
Lymphatic permeation				
Absent	372 (85.7)	16 (3.0)	93.8	
Present	62 (14.3)	5 (8.1)	90.1	0.197
Vascular invasion				
Absent	369 (85.0)	12 (3.3)	95.2	
Present	65 (15.0)	9 (13.8)	83.7	0.006[a]

[a]Indicates significance.
CEA, preoperative serum carcinoembryonic antigen level, normal upper limit at 5 ng/ml.

TABLE 4. Multivariate Analysis of Risk Factors for Late Recurrence Beyond 5 yr

Factors	Unfavorable	Favorable	Hazard Ratio	95% CI	*p*
Smoking habits	Ever-smoker	Nonsmoker	2.166	0.689–6.815	0.186
Histologic type	Squamous cell carcinoma	Nonsquamous cell carcinoma	1.612	0.567–4.584	0.370
Histological differentiation	Moderately/poorly differentiated	Well differentiated	1.318	0.436–3.982	0.625
Vascular invasion	Present	Absent	2.742	1.058–7.108	0.038[a]

[a]Indicates significance.
CEA, preoperative serum carcinoembryonic antigen level, normal upper limit at 5 ng/ml; CI, confidence interval.

recurrence rates per year peaked in the second year after resection and continued at a higher rate of 2 to 10% beyond 5 years until 9 years after resection (Figure 3). The recurrence-free probability after the first 5 years for patients with vascular invasion was significantly higher than that for patients without vascular invasion ($p < 0.001$). These findings indicate that in patients with stage IA NSCLC without vascular invasion, 5 years may be sufficient to declare that the patients are cured. In contrast, patients with stage IA NSCLC with vascular invasion need follow-up until at least 9 years after resection.

This study was retrospectively performed, and there was limitation in the analysis. We only focused on late recurrence risk beyond 5 years after resection and failed to analyze subsequent interventions and their outcomes after recurrent NSCLC diagnosis. The current study shows that cancer recurrence still occurs after 5 years although at a lower annual rate than during

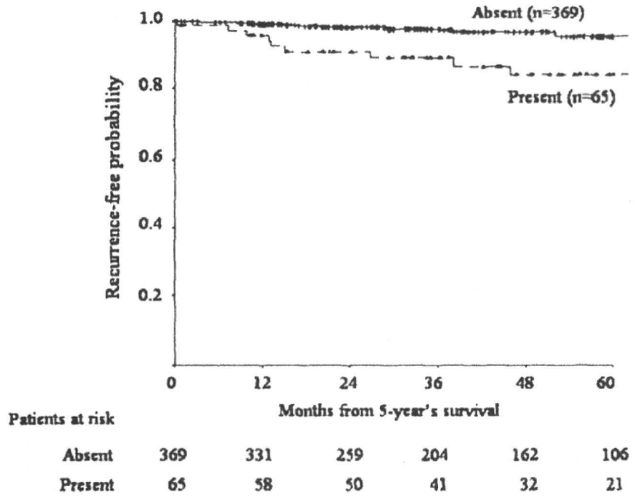

FIGURE 1. Recurrence-free probability curves from the point of 5 years after resection according to intratumoral vascular invasion.

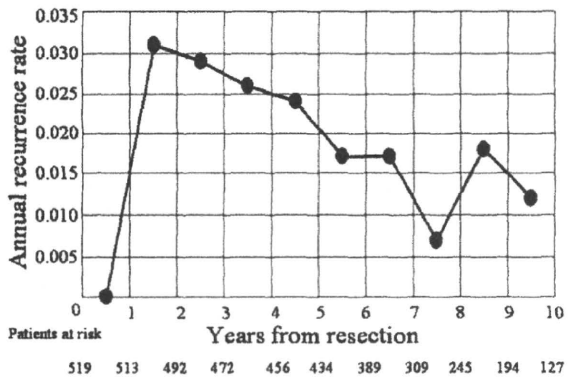

FIGURE 2. Annual recurrence rates after resection in the entire cohort.

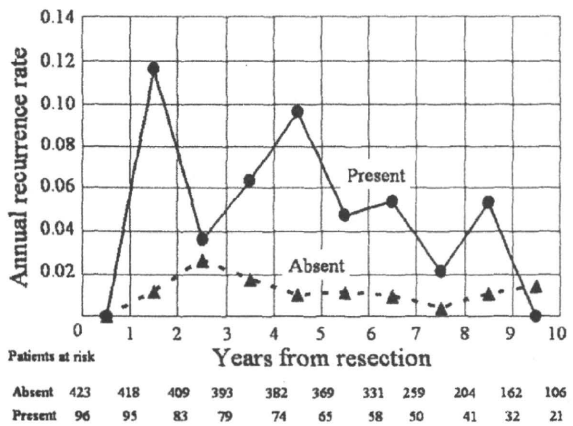

FIGURE 3. Annual recurrence rates after resection according to intratumoral vascular invasion.

the first 5 years. This may justify further surveillance, but patient benefit from identifying recurrence by annual follow-up beyond 5 years after resection was unclear.

In conclusion, 4.8% of patients with resected stage IA NSCLC who remained recurrence-free for the first 5 years later developed recurrence. In patients with stage IA NSCLC with vascular invasion, 5 years without recurrence is not sufficient to conclude that NSCLC is cured. In contrast, patients without vascular invasion may be declared to be cured at 5 years after resection if they are recurrence-free.

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CHEST

Original Research

LUNG CANCER

Long-term Survival and Risk Factors for Recurrence in Stage I Non-small Cell Lung Cancer Patients With Tumors up to 3 cm in Maximum Dimension

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

Background: **The purpose of this study was to evaluate patients with stage I non-small cell lung cancer (NSCLC) and tumors up to 3 cm in maximum dimension who underwent surgical resection on the revised TNM classification and to investigate the risk factors for recurrence.**

Methods: **Between 1994 and 2003, 713 consecutive stage I NSCLC patients with tumors up to 3 cm in maximum dimension underwent complete resection. Recurrence-free probability was estimated from the date of the primary tumor resection to the date of the first recurrence or the last follow-up using the Kaplan-Meier method.**

Results: **The recurrence-free probability of stage I NSCLC patients with tumors up to 3 cm in maximum dimension was 87% at 5 years. On multivariate analyses, three variables were shown to be independently significant recurrence risk factors: histologic differentiation (hazard ratio, 2.3), intratumoral vessel invasion (hazard ratio, 2.9), and visceral pleural invasion (VPI) (hazard ratio, 1.8). According to subgroup analyses combining these three risk factors, the 5-year recurrence-free probability was 94% for patients with zero or one factor (n = 492) and 71% for patients with two or three factors (n = 221), respectively ($P < .001$).**

Conclusion: **In stage I NSCLC patients with tumors up to 3 cm in maximum dimension, we identified three risk factors for recurrence that independently increase their risk of recurrence. In addition to VPI, histologic differentiation and intratumoral vessel invasion should be examined and their data collected for the next revision of the TNM staging system.**

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Abbreviations: CEA = serum carcinoembryonic antigen; NSCLC = non-small cell lung cancer; UFT = uracil-tegafur; UICC = International Union Against Cancer; VPI = visceral pleura invasion

In the International Association for the Study of Lung Cancer proposal for the seventh edition of the International Union Against Cancer (UICC) TNM for lung and pleural tumors,[1] T1 (< 3 cm) non-small cell lung cancer (NSCLC) tumors are subdivided into two groups according to tumor size: T1a (< 2 cm) and T1b (> 2 cm but < 3 cm). Visceral pleura invasion (VPI) is clearly defined, and T1 tumors continue to be upgraded to T2 when the visceral pleura elastic layer is invaded. However, a considerable number of otherwise small tumor patients without VPI develop recurrence, resulting in cancer death. Other than tumor size or VPI, various clinicopathologic factors in patients with small tumors have been reported to predict a poor outcome. Identifying

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Affiliations: From the Department of Thoracic Oncology (Drs Maeda, Yoshida, Hishida, Aokage, Nishimura, Nishiwaki, and Nagai), and the Department of Pathology, Research Center for Innovative Oncology (Dr Ishii), National Cancer Center Hospital East, Kashiwa, Chiba, Japan.

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Correspondence to: 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

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recurrence risk factors in these patients will help select patients who may benefit from adjuvant therapy. We reviewed a large series of consecutive stage I NSCLC patients with tumors up to 3 cm in maximum dimension resected at our hospital. The purpose of this study was to evaluate these patients based on the revised TNM classification and to investigate the risk factors for recurrence.

Materials and Methods

Patients

A total of 734 consecutive Japanese pathologic stage I NSCLC patients with tumors up to 3 cm in maximum dimension who underwent complete resection between January 1994 and December 2003 at the National Cancer Center Hospital East were identified in our departmental database. Complete resection was defined as cancer-free surgical margins both grossly and histologically. Among these patients, 21 were excluded because of (1) preoperative or postoperative chemotherapy or radiation therapy, or both (n = 6), and (2) low-grade pulmonary malignancies including carcinoids, mucoepidermoid carcinomas, or adenoid cystic carcinomas (n = 15). The remaining 713 patients were the subjects of this study.

Pathologic Evaluations

Histologic type was determined according to the World Health Organization classification,[2] and the histologic differentiation grade was categorized into well- and moderately/poorly differentiated carcinomas by a single pathologist (G. I.) who was blinded to the clinical outcome. We diagnosed squamous cell carcinoma based on the findings of keratinization, intercellular bridges, and squamous pearl formation. These features varied with degree of differentiation, being prominent in well-differentiated tumors, focal in poorly differentiated tumors, and intermediate in moderately differentiated tumors. For adenocarcinomas, bronchioloalveolar carcinoma was categorized as a well-differentiated component, acinar and papillary adenocarcinomas as moderately differentiated components, and solid carcinoma with mucin production as a poorly differentiated component. When more than one differentiation component was identified in a tumor, we registered the differentiation of the most predominant component as its histologic differentiation. We classified large cell and pleomorphic carcinomas as poorly differentiated tumors. Disease stages were based on the TNM classification of the UICC, seventh edition.[1] Intratumoral vessel invasion (ie, lymphatic permeation and vascular invasion) was evaluated by hematoxylin-eosin and Elastica-van Gieson stainings. Vascular invasion and lymphatic permeation were often analyzed separately in previous studies.[3-8] However, because differentiation between vascular invasion and lymphatic permeation can be difficult,[9] and D2-40 staining to specify lymphatic ducts was occasionally not performed in the present series, we analyzed vascular invasion and lymphatic permeation collectively as vessel invasion.

Patient Follow-up

We examined the patients on an outpatient basis at 3-month intervals for the first 2 years and typically at 6-month intervals thereafter. The follow-up evaluation included physical examination, chest radiography, and blood examination, including pertinent tumor markers. Whenever any symptoms or signs of recurrence were detected, further evaluations were performed, including CT scans of the chest and abdomen, brain MRI, and bone scintigraphy. After 2004, integrated PET and CT imaging were also performed when appropriate. We diagnosed recurrence on the basis of compatible physical examination and diagnostic imaging findings, and confirmed the diagnosis histologically when clinically feasible. The date of recurrence was defined as the date of histologic proof or, in cases diagnosed based on clinicoradiologic findings, the date of identification by a physician.

Clinicopathologic Information

We reviewed the medical records of each patient for clinicopathologic information, including age (dichotomized at the median age of 65), gender, smoking history (non- or ever-smoker), preoperative FEV_1/FVC; >70% or <70%), preoperative serum carcinoembryonic antigen (CEA) level (cut off at the normal upper limit of 5 ng/mL), extent of resection, diameter of the tumor on the resected specimen (≤ 2 cm or > 2 cm), histologic differentiation (well differentiated or moderately/poorly differentiated), intratumoral vessel invasion (presence or absence), and pleural invasion (as defined in the TNM classification, seventh edition: presence or absence).

Statistical Analysis

The length of survival was defined as the interval in months between the date of surgical resection and the date of either death or the last follow-up. The length of the recurrence-free period was calculated in months from the date of resection to the date of the first recurrence or the last follow-up. To calculate recurrence-free probability, patients who died without recurrence or who were known to be recurrence free at the date of last contact were censored. For univariate analyses, all cumulative survival rates were estimated using the Kaplan-Meier method, and differences in variables were determined using the log-rank test. Multivariate analyses were performed using Cox's proportional hazard regression model. Forward and backward stepwise procedures were used to determine independent predictors. All *P* values reported were two sided, and the significance level was set at < .05. Analyses were performed using the statistical software SPSS II for Windows, 11.0 (SPSS Inc.; Chicago, IL) and GraphPad Prism for Windows, version 5.02 (GraphPad Software, Inc.; La Jolla, CA). Data collection and analyses were approved and the need to obtain informed consent from each patient was waived by the institutional review board in August 2009.

Results

The 5- and 10-year overall survival rates of the 713 patients were 85.8% and 71.3%, respectively. Recurrence-free probability was 86.7% at 5 years and 79.7% at 10 years after resection. Table 1 lists the recurrence-free probabilities at 5 and 10 years after surgical resection according to clinicopathologic features in all patients.

Univariate analysis (log-rank test) identified nine significant risk factors for recurrence: age, gender, smoking habits, FEV_1/FVC ratio, preoperative serum CEA level, tumor diameter, histologic differentiation, intratumoral vessel invasion, and pleural invasion (Table 1). On multivariate analysis using the Cox regression model, histologic differentiation, presence

Table 1—*Recurrence-Free Probability and Clinicopathologic Characteristics*

Characteristic	No. Patients	Recurrence-Free Probability, % 5 y	10 y	Univariate *P* Value
Overall	713	86.7	79.7	
Age, y				
< 65	371	90.1	82.6	.005[a]
≥ 65	342	83	75.1	...
Gender				
Female	328	90.9	84.7	.016[a]
Male	385	83	76.5	...
Smoking habits				
Nonsmoker	318	91.3	85.8	.001[a]
Current or former smoker	395	83	75.5	...
FEV_1/FVC, %				
≥ 70	555	89	82	< .001[a]
< 70	152	78.6	73.4	...
Not performed	6	...	...	...
CEA				
Within normal range	513	89.3	82.3	.007[a]
Elevated	200	80	73.4	...
Extent of resection				
Lobectomy	616	87.4	80.5	.163
Segmentectomy or wedge resection	97	82.5	75.4	...
Tumor size, mm				
≤ 20	393	89.9	82.7	.022[a]
> 20	320	82.3	76.1	...
Histologic type				
Adenocarcinoma	569	87.9	80.8	.222
Squamous cell carcinoma	104	79.8	71.1	...
Large cell carcinoma	27	84.4	84.4	...
Adenosquamous carcinoma	9	...	...	...
Pleomorphic carcinoma	4	...	...	...
Histologic differentiation				
Well differentiated	355	95.1	90.1	< .001[a]
Moderately/poorly differentiated	358	78.5	67.8	
Intratumoral vessel invasion				
Absent	477	93.6	89.4	< .001[a]
Present	236	72.4	59.9	
Visceral pleural invasion				
Absent	605	89.5	83.8	< .001[a]
Present	108	70.2	55.6	

CEA = serum carcinoembryonic antigen level.
[a]Indicates significance.

of intratumoral vessel invasion, and presence of VPI remained statistically significant independent predictors for recurrence (Table 2). Subgroup analysis with a combination of these three independent recurrence risk factors (histologic differentiation, presence of intratumoral vessel invasion, and presence of pleural invasion for recurrence) revealed 10-year recurrence-free probabilities of 93.1%, 84.0%, 61.8%, and 42.6% for patients with zero, one, two, or three risk factors, respectively (Fig 1). The difference in recurrence-free probability was statistically significant between the zero- and one-risk-factor groups ($P < .001$) and between the one- and two-risk-factor groups ($P < .001$), but not between the two- and three-risk-factor groups ($P = .073$). When we divided the patients into two groups with either two or three factors or zero or one risk factor, the 10-year recurrence-free probabilities were 56.0% and 89.5%, respectively ($P < .001$) (Fig 2).

Of the 713 patients, 605 patients without VPI were diagnosed as stage IA based on the TNM classification of the UICC, seventh edition. Also in this subset of patients, multivariate analysis showed that histologic differentiation and presence of intratumoral vessel invasion were statistically significant independent predictors for recurrence (Table 3).

Subgroup analysis combining these two independent recurrence risk factors in stage IA patients revealed 10-year recurrence-free probabilities of 93.2%, 85.0%, and 58.9% for patients with zero, one, or two risk factors, respectively (Fig 3). The 10-year recurrence-free probability of subgroups stratified according to both T subclassification (T1a or T1b) and number of risk factors was as follows: A: T1a/zero risk factors (n = 198), 92.0%; B: T1a/one risk factor (n = 105), 86.3%; C: T1a/two risk factors (n = 54), 53.8%; D: T1b/zero risk factors (n = 93), 95.3%; E: T1b/one risk factor (n = 81), 83.8%; F: T1b/two risk factors (n = 74), 62.7% (Fig 4). The difference in recurrence-free probability stratified by the number of recurrence risk factors was statistically significant both in the T1a (A vs B and B vs C) ($P = .018$ and $P < .001$, respectively) and T1b (C vs D and D vs E) ($P = .019$ and $P = .004$, respectively) groups. In contrast, in each risk factor number group, there was no difference in survival due to T subclassification, and the recurrence-free probability curves mostly overlapped within each risk factor number group.

Discussion

Previous studies have reported several factors associated with poor prognosis in patients with small NSCLC, including tumor size,[1,3] preoperative serum CEA level,[10,11] VPI,[3,4,12] intratumoral vessel invasion,[3-6,9] and histologic differentiation.[4] Among these factors, the maximum tumor dimension is a valuable and readily available prognostic factor. Larger tumor size (> 2 cm) is a known poor prognostic factor in patients with surgically resected stage IA NSCLC. In the UICC's seventh edition of TNM for lung and pleural tumors, T1 NSCLC tumors are divided into two subgroups according to tumor size: T1a (< 2 cm) and T1b (> 2 cm but < 3 cm).[1] However, a considerable