Table 1 Histological classification of small adenocarcinoma of the lung†

Type	Description
Adenocarcinoma with replacement growth	
A	LBAC
B	LBAC with foci of collapse of alveolar structure
C′	LBAC with active fibroblastic proliferation (less than 10% of total fibrotic area)
C	LBAC with active fibroblastic proliferation (10% or more of total fibrotic area)
Adenocarcinoma with non-replacement growth	
D	Poorly differentiated adenocarcinoma
E	Tubular adenocarcinoma
F	Papillary adenocarcinoma with compressive and destructive growth (true papillary adenocarcinoma)

†Modified from reference 3. LBAC, localized bronchioloalveolar carcinoma.

examination is very useful for identifying BAC lesions and is important for assessing the prognosis of adenocarcinomas.[7,8] However, it is very difficult to adjust the diagnostic criteria based on histological features.[9–11]

The aim of the present trial was to identify an acceptable criterion that can distinguish between BAC that is *in situ* adenocarcinoma and advanced BAC or other invasive adenocarcinomas, and that general pathologists can use. We employed the diagnostic criteria for small adenocarcinoma of the lung and diagnosed 32 such cases, then assessed the efficiency and usefulness of the criteria and the effectiveness of an educational program for general pathologists.

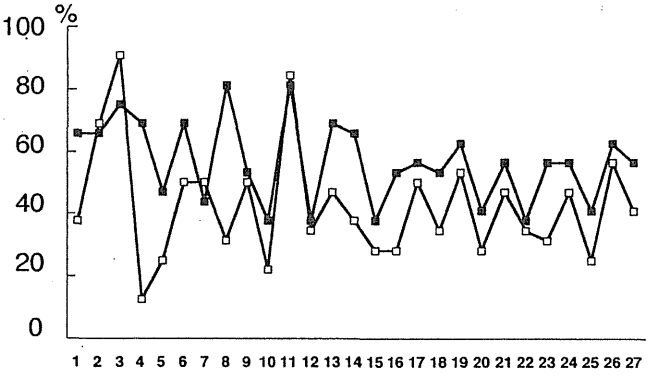

Figure 1 Diagnosis of small adenocarcinoma of the lung was compared between before and after the educational program. Coincidence rates for each participant before (□) and after (■) the educational program.

MATERIALS AND METHODS

The Lung Pathology Group of the Committee for Grants-in-Aid for Cancer Research (12-5) from the Ministry of Health, Labor and Welfare of Japan discussed the need for simple histological criteria for small adenocarcinoma of the peripheral lung and agreed on the criteria based on reported small adenocarcinomas of the lung (Table 1).[3,12] As Table 1 indicates, type A tumors show replacement growth of alveolar lining cells with minimal or mild thickening of the alveolar septa. Type B tumors are similar to type A, but contain fibrotic foci due to alveolar collapse. Type C tumors basically show a replacement growth pattern, but foci of proliferation of active fibroblasts (fibroblasts with large nuclei such as tumor cells and recognizable nucleoli) are detectable. In the present study we subdivided type C tumors into two groups according to the percentage of active fibroblasts in the total fibrotic area: type C showing ≥10%, and type C′ showing <10%. Types D, E and F tumors show non-replacement growth of the alveolar structure. Type D tumors are poorly differentiated adenocarcinoma. Type E tumors are tubular adenocarcinoma originating from, or differentiating toward, bronchial gland cells. Type F tumors are papillary carcinoma showing expansive and destructive growth.

Thirty-two small adenocarcinomas measuring <2 cm across their greatest dimensions were obtained from patients who underwent surgical resection between 1995 and 1996 at the National Cancer Center Hospital (Tokyo, Japan), University Hospital of Tsukuba (Ibaraki, Japan) and Osaka Medical Center for Cancer and Cardiovascular Diseases (Osaka, Japan). The patients selected were followed up for more than 5 years, during which time five patients died of the disease. The other patients were alive and free from cancer. The specimens were fixed with 10–15% (v/v) neutral buffered formalin at room temperature and, after being embedded in paraffin, 4 μm-thick sections were prepared and stained with hematoxylin and eosin (HE) and elastic van Gieson. Three different pathologists (MN, YMi and TI) independently examined the largest cut surface of the tumor and made diagnoses according to the diagnostic criteria for small adenocarcinoma of the lung reported by Noguchi *et al.* with some modification (Table 1).[3] Any discrepancies in the diagnosis were resolved by joint review of the slides through a multiheaded microscope. The diagnoses of the 32 tumors were type A in two cases, type B in three cases, type C′ in eight cases, type C in 13 cases, type D in four cases, and type F in two cases. The five patients who died of the cancer were diagnosed as having type C tumors.

Twenty-seven general pathologists whose major field was not lung cancer, volunteered to participate in the trial to assess the applicability of the pathological criteria and the

effectiveness of the training program for establishing mastery of the criteria and ability to diagnose small adenocarcinomas according to their prognoses. The length of experience in surgical diagnosis was <5 years for 12 pathologists and ≥5 years for 15 pathologists. The training course was held over 2 days, and before the course we asked the volunteers to diagnose the 32 cases of small adenocarcinoma according to written criteria (Table 1). After the pathologists had assessed the slides, several lectures were presented by specialists in lung cancer, including pathologists and surgeons, on the histological criteria for pulmonary adenocarcinoma and the clinical impact of histological diagnosis of these small BAC-type adenocarcinomas. The volunteers then practised reading many typical slides of small adenocarcinomas with advice from the specialists. At the end of the training program, we asked the volunteers to diagnose the same 32 lung cancers that they had diagnosed before the course started. We also asked six pathologists specializing in lung tumors to diagnose the 32 cases according to these criteria. Then we compared the coincidence rate of the histological diagnosis of the cases between the volunteers before and after the training course, and also compared the volunteers and specialists.

Statistical analysis of the correlation of the diagnostic coincidence rate was performed using Student's *t*-test. $P < 0.05$ was considered to denote statistical significance.

RESULTS

The coincidence rates of the diagnosis among the 27 participants in the first and second trials ranged from 12.5% to 90.6% and 37.5% to 81.2%, and the average rates were 42.4% and 56.6%, respectively (Table 2, Fig. 1). The average coincidence rate of each participant in the second trial was significantly higher than that in the first trial ($P < 0.01$). The coincidence rates of diagnosis among the specialists ranged from 65.6% to 81.2% and the average rate was 71.4%. The average rate was significantly higher than the average rates in the first and second trials by the participants (general pathologists; $P < 0.01$).

The distinction between *in situ* adenocarcinoma and early but invasive adenocarcinoma is very important because their prognoses are significantly different. Therefore, we divided the cases into types A and B, which were *in situ* adenocarcinomas, and types C′, C, D, E and F, which were small but invasive adenocarcinomas, and examined whether the participating pathologists could distinguish the two groups. As Table 2 shows, the average coincidence rate in the first trial was 80.3% and that in the second trial was 85.3%, and the rates were significantly different ($P < 0.05$; Fig. 2). The rate among the specialists was 89%, which was significantly higher than that of the first trial ($P < 0.05$), but not significantly different from that of the second trial.

The changes in the coincidence rates of each participant before and after the educational seminar are summarized in Figures 1,2. The coincidence rates of most participants increased after the educational program. The standard deviations of the rates decreased after the program (Table 2).

The coincidence rates for each of the cases were then examined. For seven cases (four type C′ tumors, two type C tumors and one type B tumor), the coincidence rates were <30% in the first trial. In the second trial, the coincidence rates for one type C tumor (case 13) and one type C′ tumor

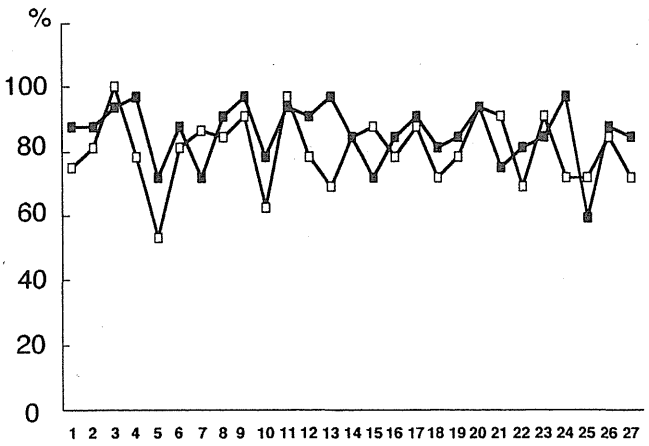

Figure 2 Discrimination between *in situ* adenocarcinoma and early but invasive adenocarcinoma was examined. Conincidence rates for each participant before (□) and after (■) the educational program.

Table 2 Coincidence rates in each trial of diagnosis

Coincidence rate (%)		MIN	MAX	AV + SD
Diagnosis of each case (types A, B, C′, C, D and F)	1	12.5	90.6	42.4* ± 17.6
	2	37.5	81.2	56.6* ± 13.2
	Sp	65.6	81.2	71.4* ± 4.90
Diagnosis of *in situ* c *vs* invasive c (types A and B *vs* types C′, C, D and F)	1	53.1	100.0	80.3** ± 10.6
	2	59.0	96.9	85.3** ± 9.10
	Sp	78.1	96.9	89.0** ± 5.90

1, first trial; 2, second trial; Sp, specialists; c, carcinoma; MIN, minimal rate; MAX, maximum rate; AV, average rate; SD, standard deviation; types A, B, C′, C, D, F: See Table 1.

*$P < 0.01$ (1 *vs* 2), $P < 0.01$ (2 *vs* Sp), $P < 0.01$ (1 *vs* Sp).

**$P < 0.05$ (1 *vs* 2), $P = 0.27$ (2 *vs* Sp), $P < 0.05$ (1 *vs* Sp).

(case 24) were still <30%. In contrast, there was one case for which the coincidence rate was >70% in the first trial, and the number increased to nine cases in the second trial. The one case in the first trial was a type D tumor. The nine cases in the second trial included one type A tumor (case 1; Fig. 3), one type B tumor (case 5), five type C tumors (cases 2, 11, 19, 20, 32) and two type D tumors (cases 10, 14; Fig. 3).

The coincidence rates of the specialists were generally higher than those of the volunteers. Although the average diagnostic rate for each case was not very high (71.4%), the average diagnostic rate for *in situ* carcinoma *vs* invasive carcinoma was nearly 90%, and all of the five patients with invasive cancer who died of the cancer were diagnosed as having invasive carcinoma by every specialist. With regard to the coincidence rates of each case, those of cases 13 and 22 were <30%. These were type C tumors and the coincidence rate for one of them (case 13) was also <30% for general pathologists. The fibrotic area of this tumor was extremely small, but active fibroblastic proliferation was detectable (Fig. 4).

The cancers examined included five type C tumors from patients who died of the cancer (case 3, 8, 11, 20 and 29). The coincidence rate of the diagnoses of these cases was 39.8%, 65.2% and 86.9% in the first and second trials and the trial by the specialists, respectively. The rate increased significantly from the first to the second trials. The diagnostic coincidence rates between *in situ* carcinoma and invasive carcinoma (types A and B *vs* types C′, C, D, E and F) were 93.8%, 98.4% and 100% in the first and second trial and the trial by the specialists, respectively. Two patients with type C tumors (cases 20 and 29) died of the cancer and were diagnosed as having type B (*in situ* adenocarcinoma) by a general pathologist even after the educational program. The cases included definite areas of fibroblastic proliferation, although the area was very limited (approximately 20% of the total fibrotic area), and a major part of the fibrotic area showed the typical alveolar collapse that is characteristic of type B tumors (Fig. 5).

DISCUSSION

The volunteers were general pathologists. Their lengths of experience varied but the educational program was very

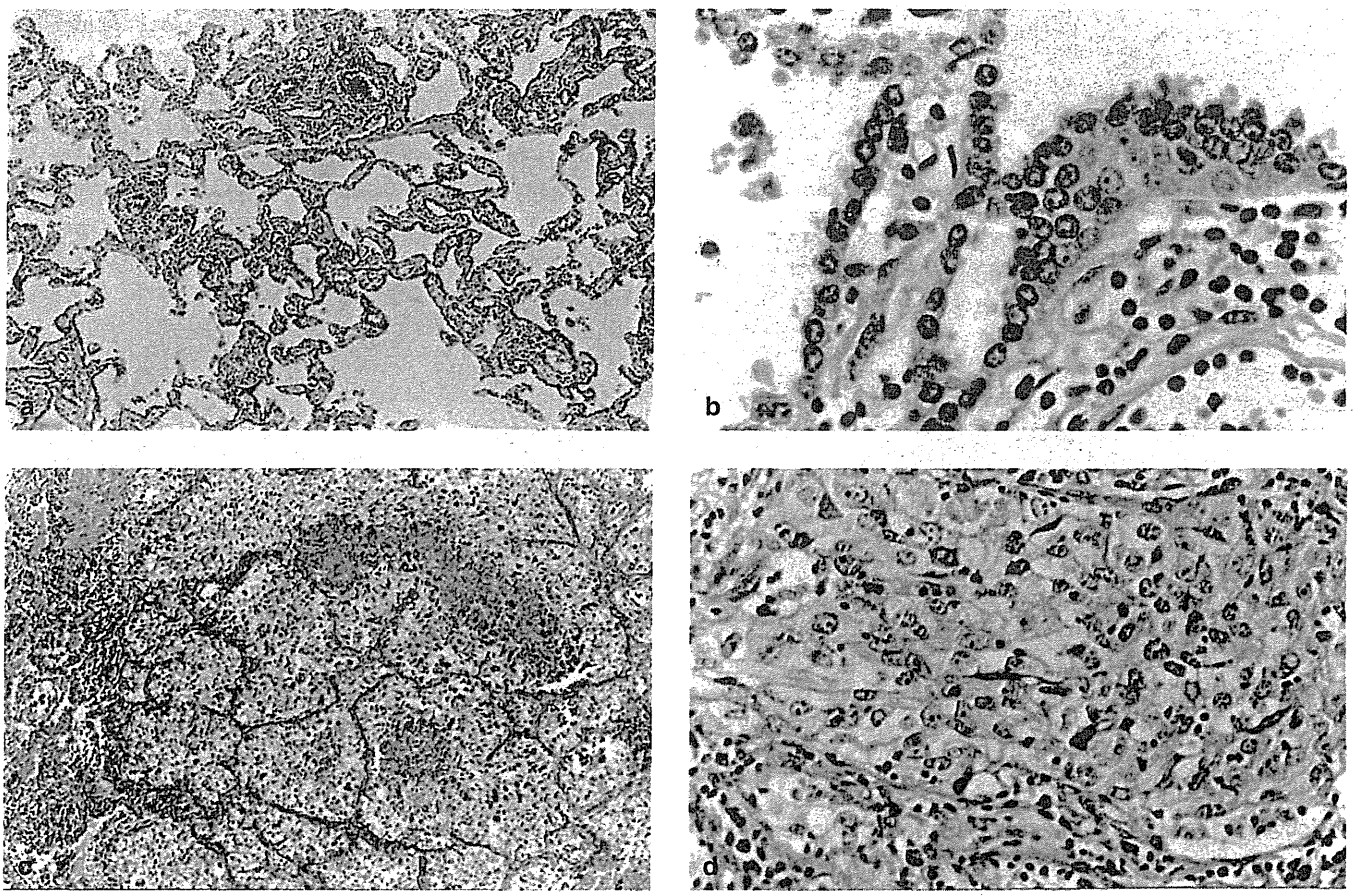

Figure 3 Histological features of (**a**,**b**) case 1 (type A) and (**c**,**d**) case 10 (type D).

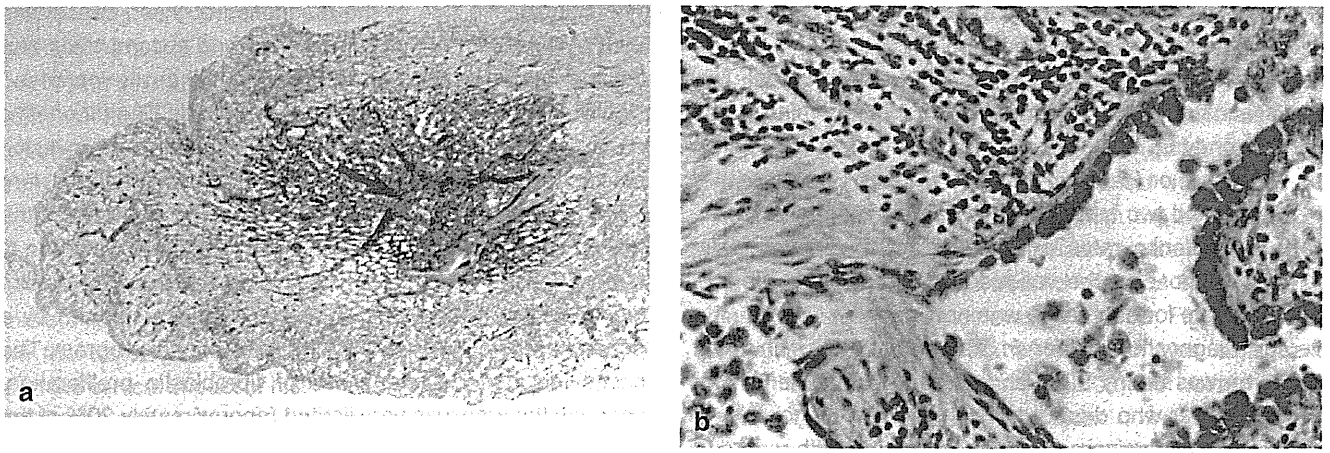

Figure 4 Whole-mount section (**a**) and histological features of case 13 (type C). Fibrotic focus in the tumor was very limited (**a**, arrow) but showed fibroblastic proliferation (**b**).

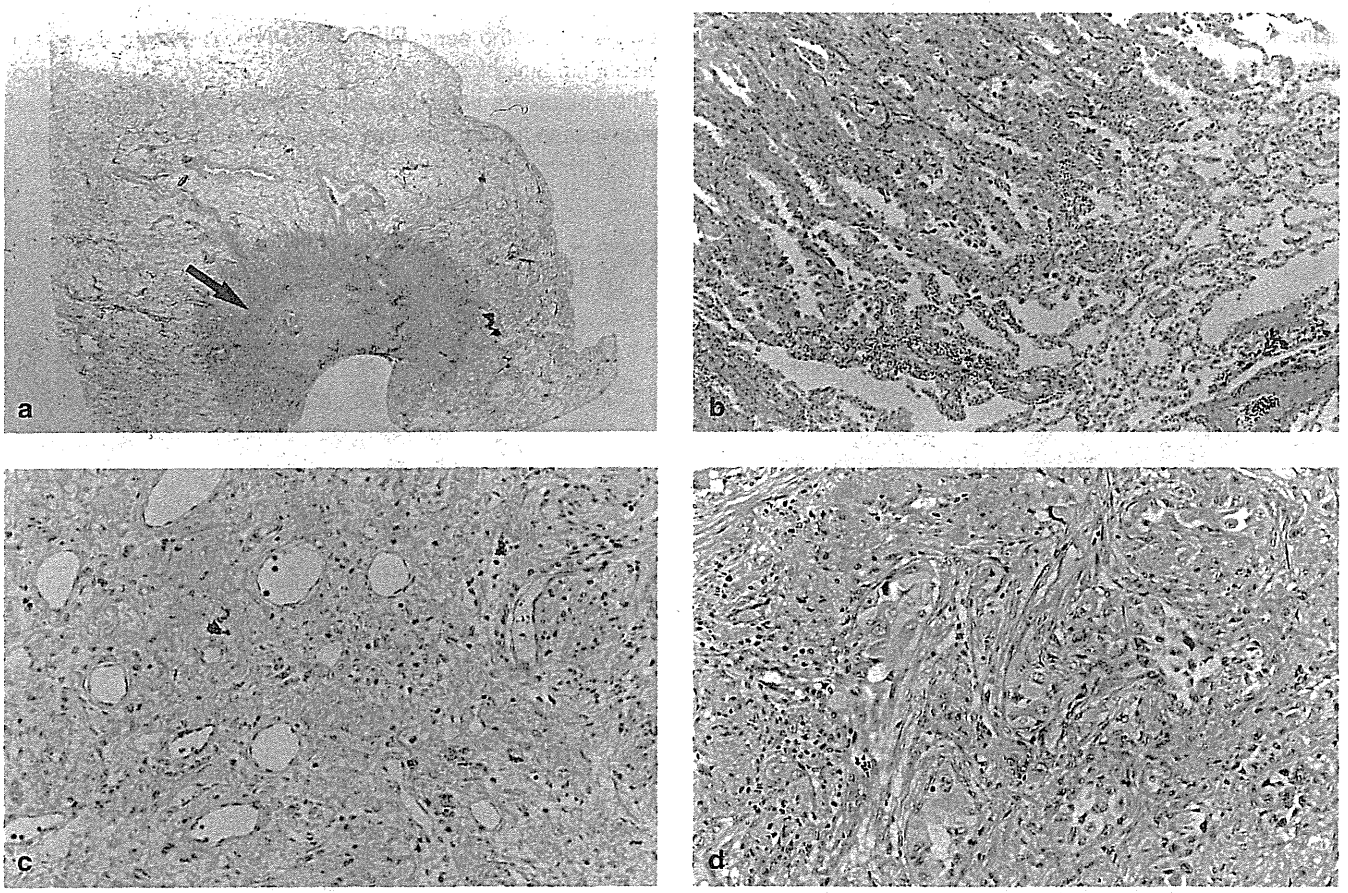

Figure 5 Whole-mount section (**a**) and histological features of case 20 (type C). Peripheral area of the tumor showed bronchioloalveolar growth (**b**) and central area showed typical collapse of the lung parenchyma (**c**). However, a very limited area (**a**, arrow) showed fibroblastic proliferation (**d**).

effective and the coincidence rates increased significantly. In particular, the distinction between *in situ* adenocarcinoma and early advanced adenocarcinoma was made at high coincidence rates after the educational program (85.3%, Table 2). The coincidence rate for the specialists was 89.0% and there were no significant differences between them.

Several cases were very difficult to diagnose. For example, there were seven cases for which the participants' coinci-

dence rate was <30% in the first trial. The coincidence rates of two cases were still under 30% in the second trial. They included type B, C′ and C tumors that had fibrotic areas (alveolar collapse) with or without areas of fibroblastic proliferation, and the structural patterns of these tumors were macroscopically similar to each other. It is unlikely that every general pathologist would have the same histological standard for assessing fibrotic foci and fibroblastic proliferation. For example, the fibrotic area of the type C tumor in case 13 was very limited, and even one of the expert pathologists failed to recognize it as a fibrotic focus (Fig. 4). In contrast, several cases were very easy to diagnose. Although only one case was diagnosed at a high coincidence rate (>70%) in the first trial, the number of such cases increased to nine in the second trial. These cases included type A and type D tumors. Type A tumor is pure bronchioloalveolar carcinoma (BAC) and type D tumor is poorly differentiated adenocarcinoma. These tumors have very characteristic structures and the participants were able to adjust to the diagnostic criteria.

The coincidence rate (*in situ* carcinoma *vs* early invasive adenocarcinoma) for the tumors of the five patients who died of the cancer was 100% for the specialists, but it is very important to note that the rate for the general pathologists was <100%. The two type C tumors of the patients who died of the cancer (cases 20 and 29) were still diagnosed as type B tumor by one general pathologist after the educational program. This pathologist seemed to neglect the area of definite fibroblastic proliferation because of its small area and the typical alveolar collapse that was a major component of the fibrotic area (Fig. 5).

Although the long-term effectiveness of this educational program was not examined, the educational program for small adenocarcinoma of the lung seemed to be very effective in establishing the diagnostic criteria that discriminate *in situ* adenocarcinoma, which has an extremely favorable prognosis (100% 5 year survival rate), from early but advanced adenocarcinoma that results in death. Histopathological diagnosis is performed based on the prognoses of each disease. This trial provided a theoretical background for the histological diagnosis of peripheral-type adenocarcinoma of the lung and supported the choice of diagnostic criteria.

ACKNOWLEDGMENTS

We thank the 27 general pathologists who participated in this trial. We also thank the six specialists in lung pathology (Dr Takayuki Honda, Shinshu University; Dr Kouki Inai, Hiroshima University; Dr Yoichi Kameda, Kanagawa Cancer Center; Dr Arafumi Maeshima, National Cancer Center Hospital; Dr Masayuki Mano, Osaka Medical Center for Cancer and Cardiovascular Diseases; Dr Tomoyuki Yokose, National Cancer Center East). This work was supported in part by a Grant-in-Aid for Cancer Research (12-5) from the Ministry of Health, Labor and Welfare of Japan and the Japanese Pathology Society.

REFERENCES

1 Travis WD, Colby TV, Corrin B, Shimosato Y, Brambilla E. *World Health Organization International Histological Classification of Tumours. Histological Typing of Lung and Pleural Tumors*, 3rd edn. Berlin: Springer and Verlag, 1999; 36–8.

2 Brambilla E, Travis WD, Colby TV *et al.* The new World Health Organization classification of lung tumours. *Eur Respir J* 2001; **18**: 1059–68.

3 Noguchi M, Morikawa A, Kawasaki M *et al.* Small adenocarcinoma of the lung. Histologic characteristics and prognosis. *Cancer* 1995; **75**: 2844–52.

4 Silver SA, Askin FB. True papillary carcioma of the lung: a distinct clinicopathologic entity. *Am J Surg Pathol* 1997; **21**: 43–51.

5 Aoyagi Y, Yokose T, Minami Y *et al.* Accumulation of losses of heterozygosity and multistep carcinogenesis in pulmonary adenocarcinoma. *Cancer Res* 2001; **61**: 7950–54.

6 Iijima T, Minami Y, Nakamura N *et al.* MMP-2 activation and stepwise progression of pulmonary adenocarcinoma: analysis of MMP-2 and MMP-9 with gelatin zymography. *Pathol Int* 2004; **54**: 295–301.

7 Kondo T, Yamada K, Noda K *et al.* Radiologic–prognostic correlation in patients with small pulmonary adenocarcinomas. *Lung Cancer* 2002; **36**: 49–57.

8 Aoki T, Tomoda Y, Watanabe H *et al.* Peripheral lung adenocarcinoma: correlation of thin-section CT findings with histologic prognostic factors and survival. *Radiology* 2001; **220**: 803–9.

9 Jacques SM, Qureshi F, Munkarah A *et al.* Interinstitutional surgical pathology review in gynecologic oncology. I. Cancer in endometrial curettings and biopsies. *Int J Gynecol Pathol* 1998; **17**: 36–41.

10 Fourcar E. 'Individuality' in the specialty of surgical pathology. Self-expression or just another source of diagnostic error? *Am J Surg Pathol* 2000; **24**: 1573–6.

11 Troxel DB. Diagnostic errors in surgical pathology uncovered by a review of malpractice claims. Part I. General considerations. *Int J Surg Pathol* 2000; **8**: 161–3.

12 Suzuki K, Yokose T, Yoshida J *et al.* Prognostic significance of the size of central fibrosis in peripheral adenocarcinoma of the lung. *Ann Thorac Surg* 2000; **69**: 893–7.

Lung Cancer (2005) 48, 339–348

ELSEVIER

LUNG CANCER
IASLC

www.elsevier.com/locate/lungcan

Prognostication of small-sized primary pulmonary adenocarcinomas by histopathological and karyometric analysis

Yuko Minami[a,b], Yoshihiro Matsuno[c], Tatsuo Iijima[b], Yukio Morishita[d], Masataka Onizuka[a], Yuzuru Sakakibara[a], Masayuki Noguchi[b,*]

[a] *Department of Surgery, Institute of Clinical Medicine, University of Tsukuba, 1-1-1 Tennodai, Tsukuba-shi, Ibaraki 305-8575, Japan*
[b] *Department of Pathology, Institute of Basic Medical Sciences, University of Tsukuba, 1-1-1 Tennodai, Tsukuba-shi, Ibaraki 305-8575, Japan*
[c] *Clinical Laboratory Division, National Cancer Center Hospital, Tsukiji 5-1-1, Chuo-ku, Tokyo 1040045, Japan*
[d] *Department of Clinical Pathology, Institute of Clinical Medicine, University of Tsukuba, 1-1-1 Tennodai, Tsukuba-shi, Ibaraki 305-8575, Japan*

Received 2 August 2004; received in revised form 18 November 2004; accepted 23 November 2004

KEYWORDS
Small-sized pulmonary adenocarcinoma;
Maximal nuclear diameter;
Fibroblastic proliferation to fibrosis area ratio;
Prognosis;
5-year relapse-free survival

Summary To reveal useful prognostic factors in cases of small-sized pulmonary adenocarcinoma, we conducted a histological and karyometric analysis of 116 small-sized pulmonary adenocarcinomas measuring less than 2 cm in maximum diameter and four specimens of atypical adenomatous hyperplasia (AAH). The small-sized pulmonary adenocarcinomas were classified by using criteria described previously [Noguchi M, Morikawa A, Kawasaki M, et al. Small adenocarcinoma of the lung. Histologic characteristics and prognosis. Lung Cancer 1995:75;2844–52]. There were 99 tumors of replacement-type adenocarcinoma, comprising 11 type A, localized bronchioloalveolar adenocarcinoma (LBAC); 6 type B, LBAC with alveolar collapse; and 82 type C, LBAC with foci of fibroblastic proliferation. The 17 remaining tumors were non-replacement-type adenocarcinomas. Among the potential prognostic factors examined, histological subtype was the most closely correlated with 5-year relapse-free survival rate. Furthermore, in patients with type C adenocarcinomas, a small fibroblastic proliferation (*F*) to fibrosis area (*f*) ratio (*F–f* ratio) (<10%) of the tumor and a small maximum nuclear diameter (Max ND; <13.50 μm) of tumor cells were closely associated with an excellent prognosis. Histological subtypes of type A and B adenocarcinomas, a small *F–f* ratio, and a small Max ND of type C adenocarcinomas were closely correlated with an excellent prognosis in small-sized adenocarcinoma.

* Corresponding author. Tel.: +81 29 853 3750; fax: +81 29 853 3150.
E-mail address: nmasayuk@md.tsukuba.ac.jp (M. Noguchi).

0169-5002/$ – see front matter © 2004 Elsevier Ireland Ltd. All rights reserved.
doi:10.1016/j.lungcan.2004.11.016

1. Introduction

Lung carcinoma is one of the commonest causes of cancer death worldwide, including Japan, and the mortality rate from this disease continues to increase [1]. Among the histological types of lung carcinoma, adenocarcinoma is that most frequently encountered in many countries [2,3]. In 1999, the World Health Organization (WHO) revised the classification of lung tumors, and newly included atypical adenomatous hyperplasia (AAH) as a pre-invasive lesion of adenocarcinoma.

In 1995, Noguchi et al. [4] classified small-sized tumors of peripheral pulmonary adenocarcinoma measuring less than 2 cm in maximum diameter into two groups, namely those showing replacement-type growth and those showing non-replacement-type growth. Each group was again subdivided into three subtypes. The replacement-type adenocarcinoma were divided into type A (localized bronchioloalveolar carcinoma, LBAC), type B (LBAC with alveolar collapse), and type C (LBAC with foci of active fibroblastic proliferation), and the non-replacement-type adenocarcinoma were divided into type D (poorly differentiated), type E (tubular growth pattern), and type F (true papillary growth pattern). Type A or B adenocarcinomas are known to carry an excellent prognosis, and the 5-year survival rate after surgical treatment has been reported to be 100%. These subtypes of adenocarcinoma are therefore biologically considered to fall under the category of carcinoma in situ and they correspond to the entity of "bronchioloalveolar carcinoma" defined by the new WHO classification [2]. On the other hand, the prognosis of type C adenocarcinoma, which is also a replacement-type adenocarcinoma, is quite poor, with a 5-year survival rate of less than 75% [4,5]. Therefore, type C adenocarcinoma is considered to be an early invasive carcinoma. Patients with this subtype of tumor are known to fall into two subsets carrying either an excellent or a poor prognosis.

Recently, examination by high-resolution computed tomography (HRCT) has come into wide use [6,7] HRCT imaging has been reported to be useful for accurate identification of each of the subtypes of small-sized pulmonary adenocarcinoma. For example, type A adenocarcinoma can be visualized as an area of ground glass opacity (GGO) and type C adenocarcinoma as an area of partial GGO on HRCT [8,9] In other words, when HRCT of an adenocarcinoma reveals GGO, a replacement-type adenocarcinoma can be suspected. Furthermore, if the GGO occupies the entire area of the nodule, type A adenocarcinoma, which carries an excellent prognosis, should be strongly suspected [9–12].

Limited resection is currently the surgical procedure of choice for type A or B adenocarcinomas, because of their excellent prognosis [13–15]; however, these tumors are detected preoperatively in only an extremely limited number of cases. If patients with type C adenocarcinoma that has excellent prognosis could be distinguished from those with the same subtype with a poor prognosis, the number of limited resections for pulmonary adenocarcinoma would be expected to increase. Therefore, it is important for us to be able to distinguish between these two groups of patients.

Our aim was to analyze surgically resected AAH and small-sized adenocarcinoma karyometrically and histopathologically, in order to extract useful prognostic factors that could be correlated with the 5-year relapse-free survival rate.

2. Materials and methods

2.1. Patients

We enrolled 119 patients (55 females and 64 males; average age, 61.3 years [range, 20–85 years]) in the study during the period from January 1995 to December 1996. The patients were from four different Japanese hospitals: the University Hospital of Tsukuba in Ibaraki, the National Cancer Center Hospital in Tokyo, the Osaka Medical Center for Cancer and Cardiovascular Disease, and the Kanagawa Cancer Center. The patients were treated by surgical resection, and 120 tumors, including those of AAH and small-sized adenocarcinoma (measuring 2 cm or less in maximum diameter) were removed. The mean maximum tumor diameter measured in the resected specimens was 15.8 mm (range, 5–20 mm).

2.2. Pathological factors

The resected specimens were fixed with 10–15% neutral buffered formalin at room temperature, and, after being embedded in paraffin, 4 μm thick sections were prepared and stained with hematoxylin and eosin (H&E) and elastica van Gieson stains. Three different pathologists (YM, TI, and MN) independently examined the tumor sections, paying special attention to the following points: classification of small-sized pulmonary adenocarcinomas [4], presence or absence of lymph node metastasis, lymphatic permeation, vascular invasion, pleural involvement, micropapillary pattern and peribronchial invasion, fibroblastic prolifera-

Table 1 Proposed diagnostic criteria of atypical adenomatous hyperplasia

Atypical adenomatous hyperplasia (AAH) is a clearly demarcated isolated alveolar-displacing proliferative lesion of the peripheral airway epithelium, and its septa are slightly thicker than normal alveolar walls. The lesions are generally no more than 5 mm in size, but at times large lesions may also be found. They are some times associated with collapsed foci within the lesions, lymphocytic infiltration, or follicle formation	
However, in cases in which it would seem impossible to differentiate between AAH and cancer, if the lesion fulfills three or more of the following five histological criteria, it may be deemed as being at least carcinoma in situ	
1	Marked cell stratification
2	High cell density, and marked overlapping of nuclei
3	Coarse nuclear chromatin, and presence of nucleolus
4	Tumor cells growing in a wooden-peg-like arrangement, or in a true papillary pattern
5	Tumor cell height greater than the height of the epithelial cells in the surrounding terminal bronchioles

tion to fibrosis area ratio, and pathological stage. Each of the pathological factors examined was defined according to the criteria given below.

Classification of AAHs and small-sized adenocarcinomas: tumors were classified according to the classification of small-sized adenocarcinomas, i.e. tumors measuring less than 2 cm in maximum diameter, as reported previously [4]. The histological definition of AAH adopted was based on the criteria shown in Table 1.

Lymph node metastasis, pathological stage: these were judged according to the TNM classification [2].

Lymphatic permeation and vascular invasion: both factors were rated as Grade 0 (non-invasion), Grade 1 (mild invasion), Grade 2 (moderate invasion), or Grade 3 (severe invasion).

Pleural involvement: pleural involvement was categorized histologically as follows: p0, tumor with no pleural involvement; p1, tumor extending beyond the elastic layer of the visceral pleura but not extending to the pleural surface; p2, tumor extending to the pleural surface but not involving the parietal pleura; and p3, tumor involving the parietal pleura or extending to organs adjacent to the lung [16].

Micropapillary pattern: a micropapillary pattern with tufts represents papillary growth without a central fibrovascular core and was judged on the basis of the criteria of Miyoshi et al. [17]. The micropapillary pattern and peribronchial invasion were categorized as Grade 0 (none), Grade 1 (mild), and Grade 2 (moderate to severe).

Fibroblastic proliferation (*F*) to fibrosis area (*f*) ratio (*F—f* ratio): The percentage area of active fibroblasts in the total fibrotic area was scored as Grade 0 (none), Grade 1 (less than 10%), Grade 2 (10—50%), or Grade 3 (more than 50%). The *F—f* ratio was examined especially in type C adenocarcinomas (Fig. 1).

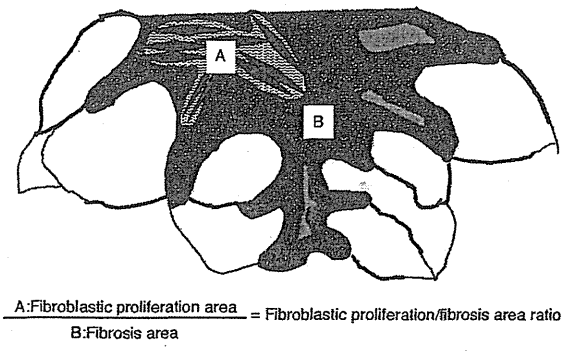

Fig. 1 Schematic diagram of calculation of fibroblastic proliferation (*F*) to fibrosis area (*f*) ratio (*F—f* ratio): the percentage area of active fibroblasts relative to the total area of fibrosis.

2.3. Karyometric analysis

An Image Processor for Analytical Pathology (IPAP: Sumika Technoservice Co. Ltd., Hyogo, Japan) was used for karyometric analysis of nuclear size, nuclear roundness, nuclear diameter, and nuclear perimeter. Fifty to sixty nuclei of the adenocarcinoma cells in each specimen were measured with computer software (IPAP-WIN Ver. 3.0, Sumika Technoservice Co. Ltd., Hyogo, Japan).

2.4. Statistical analysis

Statistical analysis of the correlations among the karyometric values in each type of adenocarcinoma was performed by Student's *t*-test. A probability value of less than 0.05 was considered to

denote statistical significance. The survival curves of the patients were drawn by the Kaplan–Meier method, and the curves were evaluated by the log-rank test or chi-squared test. The independent prognostic factors of all the small-sized pulmonary adenocarcinomas were evaluated by multivariate analysis.

3. Results

3.1. Clinical and histological findings in small-sized pulmonary adenocarcinomas

Three pathologists (YM, TI, MN) subdivided the 120 tumors into AAH and 6 subtypes of small-sized pulmonary adenocarcinoma. The 120 tumors included 4 AAHs, 11 type A adenocarcinomas, 6 type B adenocarcinomas, 82 type C adenocarcinomas, 10 type D adenocarcinoma, 3 type E adenocarcinomas, and 4 type F adenocarcinomas (Fig. 2). The male to female ratio was lower among patients with AAH, type A or type B (0.3125) than among patients with types D, E, or F (3.25) (Table 2). Ninety-three tumors were pathological stage I, 10 were stage II, 7 were stage III, and 6 were stage IV (Table 3). We could not determine the pathological stages for the other four patients, since they have taken lobectomy without lymph nodes dissection.

3.2. Pathological findings and outcome

The clinical and histological characteristics of the small-sized adenocarcinomas examined are summarized in Table 2. The 5-year relapse-free survival rate was 100% for AAH and types A and B adenocarcinoma, 80.5% for type C adenocarcinoma, and 70.6% for types D, E and F adenocarcinoma ($P=0.0207$) (Fig. 3) (Table 2). The prognosis for each pathological stage is shown in Fig. 4. The results for each pathological factor are shown in Tables 2 and 3. As can be seen from Table 3, the most significant prognostic factors for all the subtypes of small-sized pulmonary adenocarcinoma were pathological stage, and the presence or absence of lymph node metastasis, lymphatic permeation, vascular invasion, a micropapillary pattern, and peribronchial invasion. We performed a multivariate analysis to determine the factors contributing most significantly to the 5-year relapse-free survival rate of patients (Table 4). The histological subtype of the tumor bore the closest correlation to the 5-year relapse-free survival rate in cases of small-sized pulmonary adenocarcinoma.

Type C tumor was a major histological subtype of small-sized pulmonary adenocarcinoma: it was visualized as partial GGO on HRCT, and patients with this subtype fell into two subsets carrying either an excellent or a poor prognosis. We thus examined the prognostic factors in type C adenocarcinomas separately, and found that the most significant prognostic factors were pathological stage, presence or absence of lymph node metastasis, lymphatic permeation, vascular invasion, a micropapillary pattern and peribronchial invasion, and the *F–f* ratio (Table 3). Among patients with type C adenocarcinoma, the *F–f* ratio in the tumor was less than 10 % in all of those (100%) showing 5-year relapse-free survival (Fig. 5).

3.3. Karyometric analysis and outcome

We performed a karyometric analysis on the 120 resected tumors. As controls, 60 lymphocytes in the specimen of each two cases were also analyzed by IPAP. Table 5 shows the karyometric values of each subtype of small-sized pulmonary adenocarcinoma. The average, maximum (Max), and minimum (Min) nuclear diameter (ND) of the cells comprising the AAH tumors were significantly smaller than those of the type A adenocarcinoma cells. However, the standard deviation of the NDs of the cells comprising the AAH tumors and type A adenocarcinoma showed no statistically significant difference ($P=0.191$). All of the above parameters of the cells were significantly smaller in the AAH tumors and in type A and B adenocarcinomas than in type C adenocarcinoma.

The Max ND in surviving patients with type C adenocarcinoma without relapse was significantly smaller than that in those who died or developed relapse (Table 6). The average and standard deviation were also significantly different between the two groups.

Among the factors related to 5-year relapse-free survival rate in patients with type C adenocarcinoma, Max ND was the most useful and practical for distinguishing between subsets with good and poor prognoses. When we divided type C adenocarcinomas into two groups according to the prognosis, the range from 10.90 to 20.00 μm of Max ND was the significant of all values in distinguishing between the two groups (Fig. 6). For example, a Max ND of less than 13.50 μm, which is about three times as large as the average ND of a small lymphocyte, was significantly associated with a good prognosis. The 5-year relapse-free survival rate of patients whose tumor cells had a Max ND of less than 13.50 μm was 91.3%. On the other hand, the corresponding survival rate

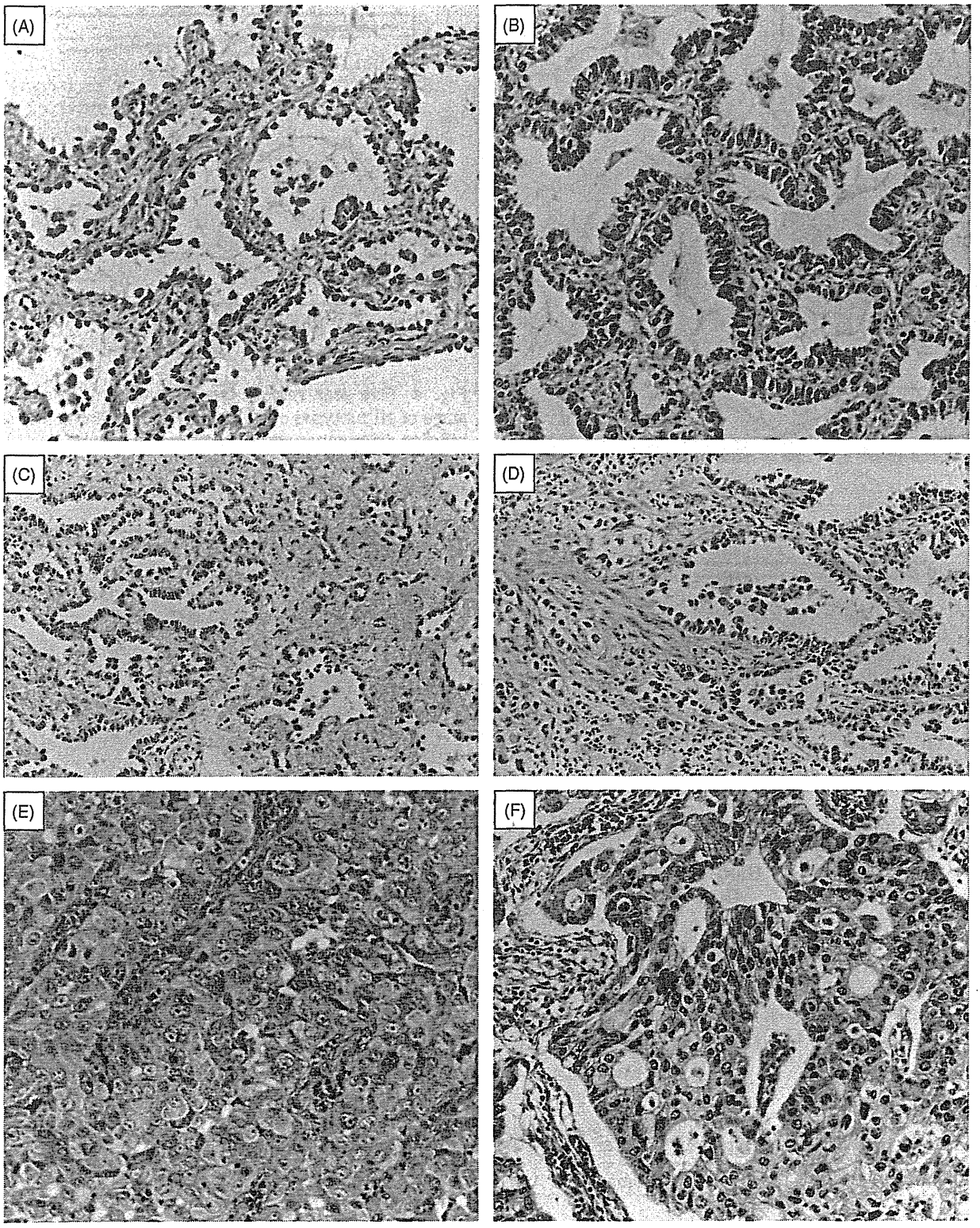

Fig. 2 A (H&E stain, 100×): histology of atypical adenomatous hyperplasia; B (H&E stain, 100×): histology of type A adenocarcinoma: localized bronchioloalveolar carcinoma (LBAC); C (H&E stain, 100×): histology of type B adenocarcinoma: LBAC with foci of collapsed alveolar septa; D (H&E stain, 100×): histology of type C adenocarcinoma: LBAC with foci of active fibroblastic proliferation; E (H&E stain, 100×): histology of type D adenocarcinoma: poorly differentiated adenocarcinoma; F (H&E stain, 100×): histology of type E adenocarcinoma: tubular adenocarcinoma; G (H&E stain, 100×): histology of type F adenocarcinoma: true papillary adenocarcinoma.

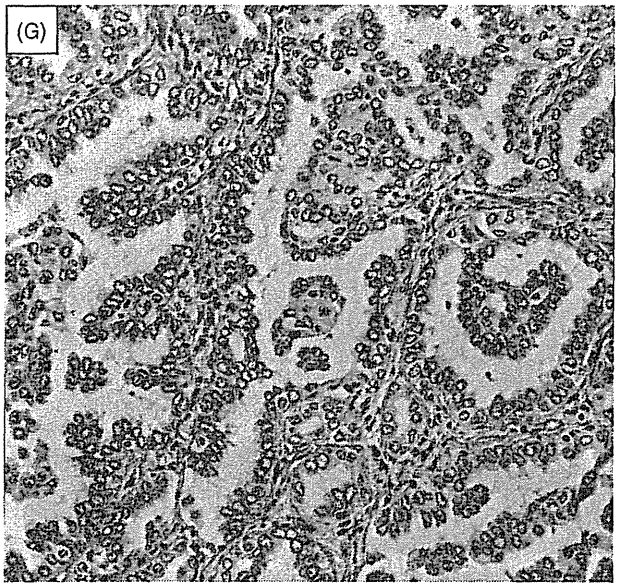

Fig. 2 (*Continued*).

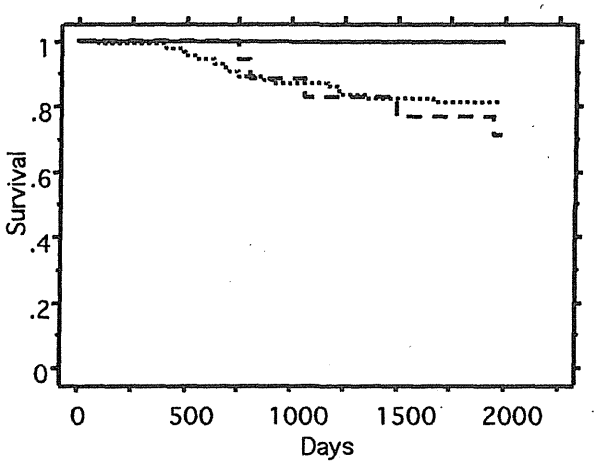

Fig. 3 Five-year relapse-free survival rates for each subtype of small-sized adenocarcinoma. Significant differences were noted between rates for each histological subtype. ($P = 0.0207$) (—); AAH and types A or B ($n = 21$), (...); type C ($n = 82$), (- - -); types D, E, or F ($n = 17$).

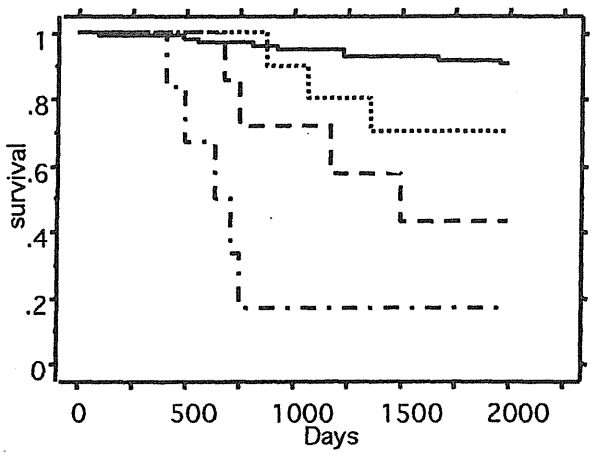

Fig. 4 Five-year relapse-free survival rates for each stage of all subtypes of small-sized adenocarcinoma. Significant differences were noted between rates for each stage. ($P < 0.0001$) (—); stage I ($n = 93$), (...); stage II ($n = 10$), (- - -); stage III ($n = 7$), (-·-·-); stage IV ($n = 6$).

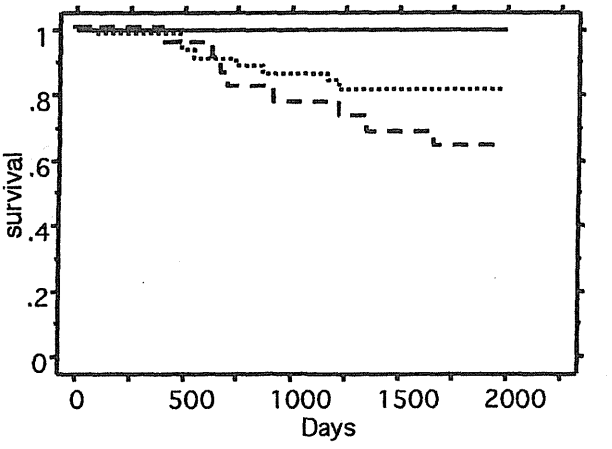

Fig. 5 Five-year relapse-free survival rates of patients with type C adenocarcinoma classified by the fibroblast to fibrosis area ratio. Significant differences were noted between rates for each category. ($P = 0.0154$) (—); fibroblast to fibrosis area ratio less than 10% ($n = 18$), (...); fibroblast to fibrosis area ratio 10—50% ($n = 42$), (- - -); fibroblast to fibrosis area ratio more than 50% ($n = 22$).

Table 2 Clinical and histological characteristics of the small-sized adenocarcinoma

Subtype	Total number of tumors	M/F[a] (M/F ratio)	Alive with relapse and dead (%)
AAH	4	2/2 (1.0)	0
A	11	2/9 (0.22)	0
B	6	1/5 (0.20)	0
Subtotal	21	5/16 (0.3125)	0
C	82	46/36 (1.27)	16 (19.5)
D, E and F	17	13/4 (3.25)	5 (29.4)
Total	120	64/56 (1.14)	21 (17.5)

[a] M/F: male/female.

Table 3 The clinico-pathological factors in each of the subtypes of small-sized adenocarcinoma

	Total	AAH	Type A	Type B	Type C	Types D, E and F	p^d (all types)	p^d (type C)
Lymph node metastasis[a]								
0	103 (12)	4	10	6	72 (10)	11 (2)	< 0.0001	<0.0001
1	5 (2)	0	0	0	2 (1)	3 (1)		
2	6 (5)	0	0	0	5 (4)	1 (1)		
3	2 (2)	0	0	0	1 (1)	1 (1)		
Lymphatic permeation								
0	94 (6)	4	11	6	63 (5)	10 (1)	<0.0001	<0.0001
1	15 (8)	0	0	0	13 (7)	2 (1)		
2	10 (6)	0	0	0	6 (4)	4 (2)		
3	1 (1)	0	0	0	0	1 (1)		
Vascular invasion								
0	101 (11)	4	11	6	68 (7)	12 (4)	<0.0001	<0.0001
1	10 (5)	0	0	0	8 (4)	2 (1)		
2	8 (4)	0	0	0	5 (4)	3		
3	1 (1)	0	0	0	1 (1)	0		
Pleural involvement								
0	94 (8)	4	11	6	60 (4)	13 (4)	NS[b]	NS[b]
1	11 (5)	0	0	0	9 (5)	2		
2	11 (8)	0	0	0	9 (7)	2 (1)		
3	4	0	0	0	4	0		
Micropapillary pattern								
0	83 (8)	4	10	6	53 (5)	10 (3)	0.0013	0.0075
1	21 (6)	0	1	0	18 (6)	2		
2	16 (7)	0	0	0	11 (5)	5 (2)		
Peribronchial invasion								
0	79 (9)	4	11	6	46 (5)	12 (4)	0.0035	0.0127
1	35 (10)	0	0	0	31 (8)	4 (2)		
2	6 (3)	0	0	0	5 (3)	1		
Fibroblastic proliferation/fibrosis area ratio								
0		NC[c]	NC[c]	NC[c]	0	NC[c]	NC[c]	0.0154
1					18			
2					42 (8)			
3					22 (8)			
Stage[a]								
1	93 (9)	4	10	6	62 (7)	11 (2)	<0.0001	<0.0001
2	10 (3)	0	0	0	8 (2)	2 (1)		
3	7 (4)	0	0	0	5 (3)	2 (1)		
4	6 (5)	0	0	0	5 (4)	1 (1)		

The number of dead patients and patients with relapse are set in parenthesis.
[a] Lymph node metastasis: lymph node dissection was not performed in four patients.
[b] NS: not significant.
[c] NC: not calculated.
[d] log-rank test for 5-year relapse-free survival.

of those with tumor cells having a Max ND of more than 13.50 μm was 66.7% ($P = 0.0046$) (Fig. 7). Although four patients in the former group (Max ND of tumor cells less than 13.50 μm) died, they all had mediastinal and/or supraclavicular lymph node metastases and/or the *F–f* ratio of their tumors exceeded 50%.

4. Discussion

AAH is a newly described entity that has been accepted as being a preinvasive lesion of pulmonary adenocarcinoma [2,3] However, the histological criteria for its diagnosis have not been rigorously defined, and it can sometimes be very difficult to dis-

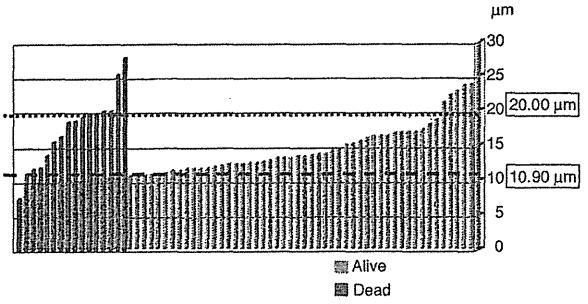

Fig. 6 The distribution of Max ND in two groups (alive and dead) of type C adenocarcinoma, the range of Max ND from 10.90 ($P=0.0489$) to 20.00 μm ($P=0.0145$) was the significant of all values in distinguishing between these two groups.

Table 4 Multivariate analysis for 5-year relapse-free survival for each of the subtypes of small-sized adenocarcinoma

Pathological factor	Coefficient of correlation
Subtype	0.56212
Lymph node metastasis	0.42701
Vascular invasion	0.37778
Peribronchial invasion	0.24700
Lymphatic permeation	0.19905
Micropapillary pattern	0.19390
Pathological stage	0.16962

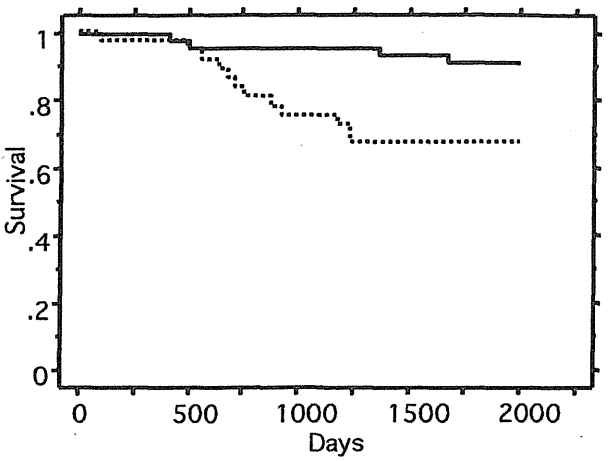

Fig. 7 Five-year relapse-free survival rates of patients with type C adenocarcinoma, classified by using the critical maximum diameter of 13.50 μm as a cut-off value. ($P=0.0046$) (—); maximum diameter less than 13.50 μm ($n=46$), (...); maximum diameter more than 13.50 μm ($n=36$).

tinguish between AAH and LBAC (type A pulmonary adenocarcinoma) [18–21]. Here, we propose appropriate and practical histological criteria for the diagnosis of AAH, and also define tumors showing a pure GGO appearance on HRCT. The results of our karyometric evaluation revealed that the average ND of cells comprising AAH was significantly smaller than that of type A adenocarcinoma cells (Table 5). The average ND of the cells comprising AAH was about 1.5 times larger than that of lymphocytes. In contrast, the same parameter in type A adenocarcinoma cells was about 1.8 times larger than that of lymphocytes. The Max ND and Min ND of cells comprising AAH and type A adenocarcinoma

Table 5 Karyometric values for each of the subtypes of small-sized adenocarcinoma

	Average N/D[a] (μm)	Maximum N/D (μm)	Minimum N/D (μm)	STDEV[b] N/D
Lymphocytes ($n=2$)	4.592 ± 0.311	5.339 ± 0.211	3.900 ± 0.031	NC[c]
AAH ($n=4$)	6.095 ± 0.304[d]	8.153 ± 0.982[d]	4.585 ± 0.153[d]	0.762 ± 0.219
A ($n=11$)	7.664 ± 0.994	10.162 ± 1.636	5.876 ± 0.691	0.954 ± 0.225
B ($n=6$)	7.717 ± 0.956	11.869 ± 2.782	5.726 ± 0.720	1.129 ± 0.335
C ($n=82$)	9.185 ± 1.601	14.364 ± 4.660	6.534 ± 0.944	1.549 ± 0.741
D, E and F ($n=17$)	9.710 ± 1.343	14.949 ± 4.346	6.950 ± 0.943	1.634 ± 0.738

[a] N/D: nuclear diameter.
[b] STDEV: standard deviation.
[c] NC: no calculation.
[d] Significantly different from type A ($p<0.01$).

Table 6 Karyometric values in cases of type C small-sized adenocarcinoma with and without good prognosis

	Average N/D[a] (μm)	Maximum N/D (μm)	Minimum N/D (μm)	STDEV[b] N/D
Type C without relapse ($n=66$)	8.916 ± 1.428[c]	13.615 ± 4.192[c]	6.419 ± 0.842	1.408 ± 0.653[d]
Type C with relapse or dead ($n=16$)	10.296 ± 1.839	17.457 ± 5.532	7.010 ± 1.201	2.126 ± 0.821

[a] N/D: nuclear diameter.
[b] STDEV: standard deviation.
[c] Significantly different from type C with relapse or dead ($p<0.05$).
[d] Significantly different from type C with relapse or dead ($p<0.01$).

were also significantly different. These results suggest that the proposed criteria would allow reasonable discrimination between AAH and type A adenocarcinoma.

We have therefore confirmed that the histological subclassification of small-sized pulmonary adenocarcinomas is useful for characterization of their clinical behavior [4]. Patients with AAH or type A or B adenocarcinoma had an extremely good prognosis (100% 5-year survival rate) compared with those classified as having type C adenocarcinoma or one of the non-replacement-type adenocarcinomas, and histological subtype proved to be the best indicator of prognosis. Our karyometric analysis revealed that the average, Max, and standard deviation of the ND were significantly different between AAH, and types A or B, and type C adenocarcinoma. The ND of type C adenocarcinoma cells was 1.5 to over three times as large as that of a lymphocyte. On the other hand, the NDs of cells comprising AAH and types A or B adenocarcinoma were the same size to twice as large as that of a lymphocyte. To distinguish between type C adenocarcinoma and AAH and type A or B adenocarcinomas, therefore, besides histological changes in the appearance of foci of active fibroblastic proliferation, nuclear diameter appears to be a powerful additional index; it is especially useful for the intraoperative diagnosis of type C adenocarcinoma.

Type C adenocarcinoma accounted for the large majority of replacement-type adenocarcinomas in our study. We identified various significant prognostic factors in small-sized pulmonary [17,22–27]. Among these factors, the *F–f* ratio was particularly significantly related to the 5-year survival of patients. MAX ND is a practically useful parameter that can be used in routine clinical laboratory testing. Between the range of 10.90 and 20.00 μm, Max NDs of 13.50 μm or 18.00 μm are useful values because they are about three or four times larger, respectively, than the average NDs of lymphocytes, and pathologists can easily estimate these sizes compared with those of lymphocytes in the same specimen.

In conclusion: (1) we have proposed practical histological criteria for recognizing AAH, with average nuclear diameter being a useful supportive criterion for distinguishing between AAH and well-differentiated adenocarcinoma; (2) histological subtype was the factor most closely correlated with the 5-year relapse-free survival rate in patients with small-sized pulmonary adenocarcinoma; (3) determination of the *F–f* ratio is useful for distinguishing between subsets of type C adenocarcinoma patients with excellent and poor prognoses; (4) the maximum nuclear diameter of tumor cells was also closely related with the 5-year relapse-free survival rate in patients with type C adenocarcinoma. It appears to be a practical and useful indicator for distinguishing between subsets of type C adenocarcinoma patients with excellent and poor prognoses.

Acknowledgements

We thank Dr. Yoichi Kameda of the Kanagawa Cancer Center and Dr. Masayuki Mano of the Osaka Medical Center for Cancer and Cardiovascular Disease for permitting us to analyze our cases of small-sized pulmonary adenocarcinoma at their respective hospitals. This study was supported in part by a Grant-in-Aid for Cancer Research (12-5) from the Ministry of Health, Labour and Welfare of Japan.

References

[1] Marugame T, Mizuno S. Mortality trend of lung cancer in Japan: 1960–2000. Jpn J Clin Oncol 2003;33:148–52.
[2] Travis WD, Colby TV, Corrin B, et al., editors. World Health Organization international histological classification of tumors. Histological typing of lung and pleural tumors. 3rd ed. Berlin: Springer; 1999.
[3] Brambilla E, Travis WD, Colby TV, et al. The new World Health Organization classification of lung tumors. Eur Respir J 2001;18:1059–68.
[4] Noguchi M, Morikawa A, Kawasaki M, et al. Small adenocarcinoma of the lung. Histologic characteristics and prognosis. Cancer 1995;75:2844–52.
[5] Kodama K, Higashiyama M, Yokouchi H, et al. Prognostic value of ground-glass opacity found in small lung adenocarcinoma on high-resolution CT scanning. Lung Cancer 2001;33:17–25.
[6] Sone S, Takashima S, Li F, et al. Mass screening for lung cancer with mobile spiral computed tomography scanner. Lancet 1998;351:1242–5.
[7] Henschke CJ, McCauley DI, Yankelevitz DF, et al. Early lung cancer action project: overall design and findings from baseline screening. Lancet 1999;354:99–105.
[8] Aoki T, Nakata H, Watanabe H, et al. Evolution of peripheral lung adenocarcinomas: CT findings correlated with histology and tumor doubling time. Am J Radiol 2000;174:763–8.
[9] Yang ZG, Sone S, Takashima S, et al. High-resolution CT analysis of small peripheral lung adenocarcinomas revealed on screening helical CT. Am J Radiol 2001;176:1399–407.
[10] Aoki T, Tomoda Y, Watanabe H, et al. Peripheral lung adenocarcinoma: correlation of thin-section CT findings with histologic prognostic factors and survival. Radiology 2001;220:803–9.
[11] Takashima S, Maruyama Y, Hasegawa M, et al. Prognostic significance of high-resolution CT findings in small peripheral adenocarcinoma of the lung: a retrospective study on 64 patients. Lung Cancer 2002;36:289–95.
[12] Takashima S, Maruyama Y, Hasegawa M, et al. High-resolution CT features: prognostic significance in peripheral lung adenocarcinoma with bronchioloalveolar carcinoma components. Respiration 2003;70:36–42.

[13] Kondo T, Yamada K, Noda K, et al. Radiologic-prognostic correlation in patients with small pulmonary adenocarcinomas. Lung Cancer 2002;36:49–57.
[14] Matsuguma H, Yokoi K, Anraku M, et al. Proportion of ground-glass opacity on high-resolution computed tomography in clinical T1 N0 M0 adenocarcinoma of the lung: a predictor of lymph node metastasis. J Thorac Cardiovasc Surg 2002;124:278–84.
[15] Ishiwa N, Ogawa N, Shoji A, et al. Correlation between lymph node micrometastasis and histologic classification of small lung adenocarcinoma, in considering the indication of limited surgery. Lung Cancer 2003;39:159–64.
[16] The Japan Lung Cancer Society, editors. General rule for clinical and pathological record of lung cancer, 6th ed. Tokyo: Kanehara & Co. Ltd.; 2003 [in Japanese with English abstract].
[17] Miyoshi T, Satoh Y, Okumura S, et al. Early-stage lung adenocarcinoma with a micropapillary pattern, a distinct pathologic marker for a significantly poor prognosis. Am J Surg Pathol 2003;27:101–9.
[18] Yokozaki M, Kodama T, Yokose T, et al. Differentiation of atypical adenomatous hyperplasia and adenocarcinoma of the lung by use of DNA ploidy and morphometric analysis. Mod Pathol 1996;9:1156–64.
[19] Morishita Y, Fukasawa M, Takeuchi M, et al. Small-sized adenocarcinoma of the lung. Cytologic characteristics and clinical behavior. Cancer (Cancer Cytopathol) 2001;93:124–31.
[20] Nakanishi K. Alveolar epithelial hyperplasia and adenocarcinoma of the lung. Arch Pathol Lab Med 1990; 114:363–8.
[21] Mori M, Chiba R, Takahashi T. Atypical adenomatous hyperplasia of the lung and its differentiation from adenocarcinoma. Characterization of atypical cells by morphometry and multivariate cluster analysis. Cancer 1993;72: 2331–40.
[22] Wu J, Ohta Y, Minato H, et al. Nodal occult metastasis in patients with peripheral lung adenocarcinoma of 2.0 cm or less in diameter. Ann Thorac Surg 2001;71:1777–8.
[23] Suzuki K, Yokose T, Nishimura J, et al. Prognostic significance of the size of center fibrosis in peripheral adenocarcinoma of the lung. Ann Thorac Surg 2000;69:893–7.
[24] Maeshima AM, Niki T, Maeshima A, et al. Modified scar grade: a prognostic indicator in small peripheral lung adenocarcinoma. Cancer 2002;95:2546–54.
[25] Asamura H, Ando M, Matsuno Y, et al. Histopathologic prognostic factors in resected adenocarcinomas. Is nuclear DNA content prognostic? Chest 1999;115:1018–24.
[26] Silver SA, Askin FB. True papillary carcinoma of the lung: a distinct clinicopathologic entity. Am J Surg Pathol 1997;21:43–51.
[27] Amin MB, Tamboli P, Merchant SH, et al. Micropapillary component in lung adenocarcinoma: a distinctive histologic feature with possible prognostic significance. Am J Surg Pathol 2002;26:358–64.

Immunohistochemical KIT (CD117) Expression in Thymic Epithelial Tumors*

Kazuo Nakagawa, MD; Yoshihiro Matsuno, MD; Hideo Kunitoh, MD; Arafumi Maeshima, MD; Hisao Asamura, MD; and Ryosuke Tsuchiya, MD

Study objectives: **It is sometimes very difficult both clinically and pathologically to distinguish thymic epithelial tumors from primary lung carcinoma with massive anterior mediastinal involvement. The expression of KIT (CD117) in thymic epithelial tumors was investigated in order to evaluate its usefulness as a marker supporting differential diagnosis and choice of therapy.**

Methods: **We examined the immunohistochemical expression of KIT in 70 resected thymic epithelial tumors (thymomas, 50; thymic carcinomas, 20) that had been reclassified on the basis of the World Health Organization histologic classification system. We also compared the expression of KIT and CD5 in 20 thymic carcinomas with their expression in 20 resected pulmonary squamous cell carcinomas that were spreading directly into the mediastinum.**

Results: **Of the 50 thymomas, only 2 (4%) showed positive immunoreactivity for KIT (type A thymoma, 1; type B3 thymoma, 1), whereas 16 of the 20 thymic carcinomas (80%) showed positive immunoreactivity. Testing was positive for CD5 in 14 of the 20 thymic carcinomas (70%). In the pulmonary squamous cell carcinomas, in contrast, the immunohistochemical expression of KIT and CD5 was found in only 4 of 20 carcinomas (20%) and 3 of 20 carcinomas (15%), respectively. Furthermore, of the 40 specimens examined (either thymic or lung carcinoma) all 13 that were positive for both KIT and CD5 were thymic carcinomas, and 13 of the 16 that were negative for both were lung carcinomas.**

Conclusion: **KIT expression is a useful immunohistochemical marker for the diagnosis of thymic carcinoma, and its examination in combination with CD5 immunohistochemistry would greatly help in the differential diagnosis of primary thymic carcinoma from pulmonary squamous cell carcinoma. Further investigations at a genetic level should be encouraged, not only to define the role of KIT in the oncogenesis of thymic epithelial tumors, but also to establish target-based therapy.** ***(CHEST 2005; 128:140–144)***

Key words: c-kit; KIT; thymic carcinoma; thymic epithelial tumor; thymoma

Abbreviations: AML = acute myeloid leukemia; GIST = GI stromal tumor; SCF = stem cell factor; SCLC = small cell lung carcinoma

It is often difficult, not only by radiographic imaging but also by pathologic examination, to distinguish thymic epithelial tumors (*ie*, thymomas or thymic carcinomas) from primary lung carcinomas of the central type involving the mediastinum.[1] However, these three tumors must be distinguished from each other because the treatment of choice is obviously different for each of them. A chance of cure may be lost by a misdiagnosis of thymoma or localized primary thymic carcinoma as advanced lung carcinoma with massive mediastinal involvement. Moreover, the thymic carcinoma shows a better response to chemotherapy or radiotherapy, and hence better patient outcome,[2–4] whereas the latter shows more frequent regional lymph node involvement and distant metastasis.

In daily practice, differential diagnosis between those two different tumors depends on several parameters that are not necessarily specific, that is, smoking history (favors lung cancer), the main location of the tumor (pulmonary hilum vs anterior mediastinum), and the presence of abundant hyalin-

*From the Departments of Pathology (Drs. Nakagawa, Matsuno, and Maeshima), Medical Oncology (Dr. Kunitoh), and Thoracic Surgery (Drs. Asamura and Tsuchiya), National Cancer Center Hospital and Research Institute, Tokyo, Japan.

This work was supported in part by Grants-in-Aid for Cancer Research (12–5, 14S-4, and 16–6) from the Ministry of Health, Labor, and Welfare of Japan.

Manuscript received May 19, 2004; revision accepted November 9, 2004.

Correspondence to: Yoshihiro Matsuno, MD, Department of Pathology, National Cancer Center Hospital, 1–1, Tsukiji 5-chome, Chuo-ku, Tokyo 104-0045, Japan; e-mail: ymatsuno@ncc.go.jp

ized collagenous stroma or abrupt keratinization simulating Hassall corpuscles (favors thymic carcinoma). Although immunoreactive CD5 has been reported[5–7] as a useful marker for primary thymic squamous cell carcinoma in routine surgical pathology, it is still necessary to identify other diagnostic markers to make this differential diagnosis more reliable.

We investigated the immunohistochemical expression of KIT (CD117) in primary thymic carcinoma. KIT, a protein product of the c-kit protooncogene, is a transmembrane tyrosine kinase receptor and is a growth factor receptor for stem cell factor (SCF).[8] Its expression has been documented in a wide variety of human neoplasms, including acute myeloid leukemia (AML), mast cell tumor, germ cell tumor, ovarian carcinoma, malignant melanoma, GI stromal tumor (GIST), small cell lung carcinoma (SCLC), neuroblastoma, and breast carcinoma.[9–15] Molecular targeted therapy against KIT has become a standard treatment of choice in patients with AML and GIST.[16] The expression of KIT in thymic epithelial tumors has been reported very recently.[17,18] We examined KIT expression in these tumors immunohistochemically and evaluated the usefulness of immunoreactive KIT as a diagnostic marker in primary thymic carcinoma.

Materials and Methods

Tumors

There were 161 resected thymic epithelial tumors in the pathology file of the National Cancer Center Hospital, Tokyo, Japan. We reviewed hematoxylin-eosin-stained sections of each specimen to determine its histologic subtype on the basis of the World Health Organization histologic classification.[19] Accordingly, 50 cases of thymomas (5 histologic subtypes, 10 each) and 20 cases of thymic carcinomas (epidermoid carcinomas, 5; nonkeratinizing epidermoid carcinomas, 11; basaloid carcinoma, 1; papillary carcinoma, 1; undifferentiated carcinoma, 1) were extracted from the files and employed in this study. Four patients with thymoma (two patients with type B2 and two patients with type B3 received preoperative chemotherapy, and three patients with thymic carcinoma received preoperative therapy; two patients underwent chemotherapy, and one patient underwent chemoradiotherapy.

In addition, 20 resected specimens of primary squamous cell carcinoma of the lung (10 well-differentiated to moderately differentiated carcinomas and 10 poorly differentiated carcinomas) involving the anterior mediastinum were also extracted and analyzed immunohistochemically for comparison. Primary lung carcinomas showing massive involvement in the anterior mediastinum were excluded from the study to avoid confusion with thymic carcinoma.

Immunohistochemistry

Immunohistochemistry was performed on representative formalin-fixed paraffin sections. The sections were autoclaved for 10 min in 10 mmol/L citrate buffer (pH, 6.0) for antigen retrieval before incubation with a primary antibody. Two monoclonal antibodies, anti-KIT (Dako; Glostrup, Denmark) and anti-CD5 (NCL-CD5–4C5; Novocastra; Newcastle-on-Tyne, UK) were used as primary antibodies. Immunoreaction was detected by a labeled streptavidin-biotin method and was visualized with 3,3′-diaminobendizine, followed by counterstaining with hematoxylin. The degree of immunostaining of those two markers was scored as follows: –, negative staining; 1+, staining (*ie*, < 10% of tumor cells stained); 2+, staining (*ie*, 10 to 50% stained); and 3+, staining (*ie*, > 50% stained). KIT staining was judged to be positive when unequivocal membranous staining was observed along the cell membrane.

Results

KIT Immunoreactivity in Thymomas

First, we studied the immunohistochemical KIT expression in thymomas (Table 1). Of the 50 thymomas examined, only 2 (4%) were positive for KIT, one of which was a type A thymoma and the other one was a type B3. Although the former specimen was classified as a type A thymoma, this tumor was composed of spindle cells with mild atypia. The other thymomas were completely negative for KIT staining in both the epithelial cells and lymphocytes.

KIT Immunoreactivity in Thymic Carcinomas and Pulmonary Squamous Cell Carcinomas

Next, we examined and compared the expression of immunoreactive KIT in thymic carcinomas and pulmonary squamous cell carcinomas. The results are presented in Table 2. Of the 20 thymic carcinomas, 16 (80%) were positive for KIT (keratinizing epidermoid [squamous cell] carcinomas, 3 of 5; nonkeratinizing epidermoid carcinomas, 10 of 11; basaloid carcinoma, 1; undifferentiated carcinomas, 2). The papillary carcinoma was negative for KIT. Thymic carcinomas that were positive for KIT exhib-

Table 1—*Expression of KIT in Thymoma**

Tumor Specimen	Tested, No.	Positive Cases, No. (%)	KIT Immunoreactivity			
			3 +	2 +	1 +	–
Thymoma	50	2/50 (4)	1	0	1	48
Type A	10	1/10 (10)	1†	0	0	9
Type AB	10	0/10 (0)	0	0	0	10
Type B1	10	0/10 (0)	0	0	0	10
Type B2	10	0/10 (0)	0	0	0	10
Type B3	10	1/10 (10)	0	0	1	9

*– = negative staining; 1 + = staining (< 10% of tumor cells); 2 + = staining (10 to 50%); 3 + = staining (> 50% of tumor cells).
†This thymoma is composed of spindle cells with mild atypia and is probably classified as an atypical thymoma, although it belongs to the type A histologic subtype in the World Health Organization classification.

Table 2—*Expression of KIT and CD5 in Thymic Carcinoma and Pulmonary Squamous Cell Carcinoma**

Tumor Type	Tested, No.	Positive Cases, No. (%)	KIT Immunoreactivity 3+	2+	1+	−	Positive Cases, No. (%)	CD5 Immunoreactivity 3+	2+	1+	−
Thymic carcinoma (type C)	20	16/20 (80)	11	3	2	4	14/20 (70)	6	7	1	6
Epidermoid, keratinizing	5	3/5 (60)	2	0	1	2	2/5 (40)	0	1	1	3
Epidermoid, nonkeratinizing	11	10/11 (91)	7	3	0	1	9/11 (80)	5	4	0	2
Basaloid	1	1/1 (100)	1	0	0	0	1/1 (100)	0	1	0	0
Papillary	1	0/1 (0)	0	0	0	1	1/1 (100)	0	1	0	0
Undifferentiated	2	2/2 (100)	1	0	1	0	1/2 (50)	1	0	0	1
Pulmonary squamous cell carcinoma	20	4/20 (20)	0	3	1	16	3/20 (15)	0	0	3	17
Well or moderately differentiated	10	2/10 (20)	0	2	0	8	1/10 (10)	0	0	1	9
Poorly differentiated	10	2/10 (20)	0	1	1	8	2/10 (20)	0	0	2	8

*See Table 1 for terms not used in the text.

ited predominantly membrane staining (Fig 1). In contrast, only 4 of 20 pulmonary squamous cell carcinomas (20%) were positive for KIT (well-differentiated or moderately differentiated carcinomas, 2; poorly differentiated carcinomas, 2).

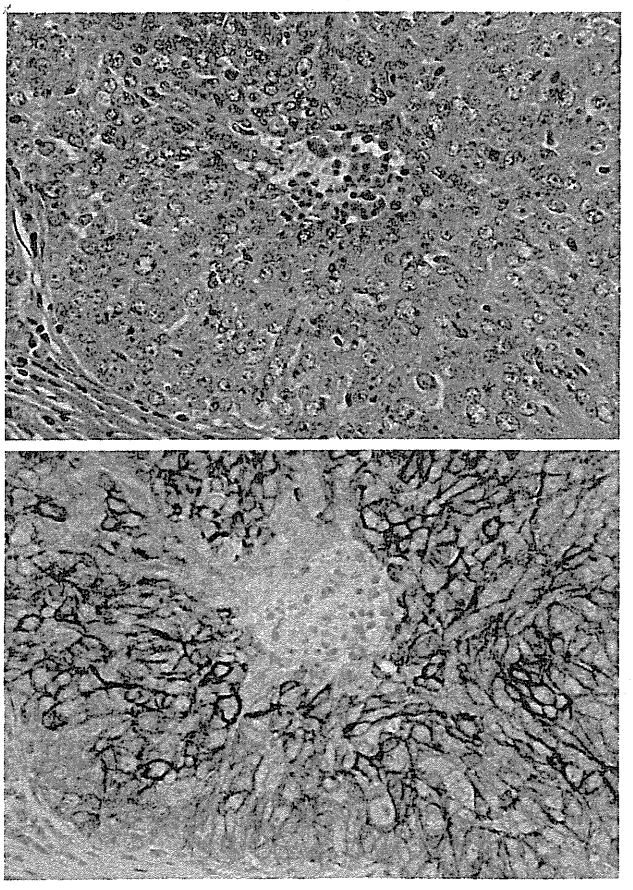

FIGURE 1. Thymic carcinoma (epidermoid nonkeratinizing carcinoma), showing diffuse membranous staining for KIT. *Top*: hematoxylin-eosin, original ×100. *Bottom*: immunoperoxidase stain, original ×100.

Comparison of CD5 Immunoreactivity in Thymic Carcinoma and Pulmonary Squamous Cell Carcinoma

Because it is widely known that CD5 is a supportive immunohistochemical marker for thymic carcinoma, we examined CD5 expression in thymic carcinoma and pulmonary squamous cell carcinoma to compare it with the results obtained for KIT. Of the 20 thymic carcinomas, 14 (70%) were positive for CD5 (keratinizing epidermoid carcinomas, 2 of 5; nonkeratinizing epidermoid carcinomas, 9 of 11; the basaloid carcinoma, 1; the undifferentiated carcinoma, 1; the papillary carcinoma, 1). In contrast, only 3 of 20 pulmonary squamous cell carcinomas (15%) were positive for CD5 (well-differentiated or moderately differentiated carcinomas, 1; poorly differentiated carcinomas, 2). The immunoreactivity for KIT in thymic carcinomas and pulmonary squamous cell carcinomas was almost equal to that for CD5. Furthermore, of the 40 specimens (either thymic or lung carcinoma) examined immunohistochemically, 13 displayed positivity for both KIT and CD5 (Table 3). All of them (100%) were thymic carcinomas. None of the pulmonary squamous cell carcinomas was positive for both markers. Of the 11 specimens that were positive for only one of the two markers, 4 specimens were thymic carcinomas and 7 were pulmonary carcinomas. Sixteen of the 40 specimens were negative for both markers. They consisted of 3 thymic carcinomas and 13 pulmonary squamous cell carcinomas.

Discussion

KIT is the protein product of the c-kit protooncogene and is immunologically identified by the CD117 antigenic epitope. Functionally, it is a transmembrane tyrosine kinase receptor the physiologic ligand of which is cytokine SCF, also called *mast cell*

Table 3—*Simultaneous Expression of KIT and CD5**

Tumor Type	KIT + CD5 +	KIT + CD5 −	KIT − CD5 +	KIT − CD5 −
Thymic carcinoma	13	3	1	3
Pulmonary squamous cell carcinoma	0	4	3	13

*n = 20.

growth factor or *Steel factor*.[8] Mainly from immunohistochemical studies[9,11,12] in human tissue, it has been postulated that KIT plays an important role in multiple cellular functions, including survival, proliferation, adhesion, differentiation, and functional maturation. KIT expression has also been reported in a wide variety of human solid tumors, such as mast cell tumors, germ cell tumors, ovarian carcinomas, malignant melanomas, GISTs, SCLCs, neuroblastomas, and breast carcinomas, and is thought to be largely implicated in the development of these tumors.[9–15] However, only a few studies[17,18] have been done to evaluate its expression in thymic epithelial tumors. We examined the immunohistochemical expression of KIT in resected thymic epithelial tumors, and demonstrated that most thymic carcinomas (80%) were positive for KIT and that most thymomas (96%) were negative for KIT. In accordance with these results, thymic carcinoma should be added to the list of KIT-positive tumors.

The following three general mechanisms of KIT activation in tumor cells have been recognized: (1) autocrine and/or paracrine stimulation of the receptor by its ligand; (2) cross-activation by other kinases and/or loss of regulatory phosphatase activity; and (3) acquisition of activating mutation.[16,20–27] In SCLC, because the coexpression of SCF and KIT has been demonstrated, autocrine mechanisms may play a role in tumor initiation and progression.[22,23] In contrast, the activation of a mutation in the *c-kit* gene is thought to be the most important factor in the pathogenesis of GISTs.[25] Moreover, elevated kinase activity caused by a *c-kit* gene mutation is now utilized as a molecular target of therapy for GISTs. In thymic epithelial tumors, as shown by our data, frequent and strong KIT expression appears to be closely associated with malignancy, because thymic carcinoma is more aggressive than thymoma and is highly lethal.[2–4] Thus, KIT must play an important role in the acquisition of a malignant phenotype in thymic epithelial tumors. Its molecular mechanism is still to be clarified.

The above discussion is obviously relevant to therapeutic strategies for thymic carcinoma. To date, no standard medical therapy has been established in the field of thymic epithelial tumors, particularly for thymic carcinoma.[2–4] If the activation of a mutation in the catalytic domain of the *c-kit* gene is the causative event in thymic carcinoma, as is the case in AML and GISTs, molecular target therapy using a specific kinase inhibitor would be indicated. Although most studies of KIT-positive human solid tumors have failed so far to identify the activating *c-kit* gene mutation, this avenue should still be investigated in thymic carcinomas in an effort to make an effective therapy available.

Shimosato and Mukai[1] noted that in poorly differentiated squamous cell carcinomas, mucoepidermoid carcinomas, small cell neuroendocrine carcinomas, clear cell carcinomas, and sarcomatoid carcinomas, the distinction between thymic carcinoma and lung carcinoma was difficult, and depended largely or entirely on the location of the primary tumor and lymph node metastasis. Differential diagnosis would be even more challenging if needle biopsy specimens were submitted. As an ancillary examination, immunoreactive CD5 is the only marker available in routine surgical pathology that supports the diagnosis of thymic carcinoma against lung carcinoma.[5–7] In our study, KIT was positive in a significant proportion of thymic carcinomas (16 of 20; 80%), whereas it was negative in most pulmonary squamous cell carcinomas (16 of 20; 80%) as one recent study shows.[28] Thus, it is reasonable to conclude that KIT is another useful immunohistochemical marker for thymic carcinoma. In combination with CD5, it should provide a more powerful tool for distinguishing between thymic carcinoma and lung carcinoma, because our data indicate that tumors that are positive for both KIT and CD5 are almost exclusively thymic carcinomas. In contrast, when a tumor is negative for both KIT and CD5, it is probably a lung carcinoma involving the mediastinum.

Precise differential diagnosis by the examination of pathologic specimens, aided by this effective examination and an elucidation of the molecular mechanisms of KIT activation, will most probably promote the establishment of a standard therapy for thymic carcinoma in the near future.

ACKNOWLEDGMENT: The authors are grateful to Ms. T. Shimizu, Clinical Laboratory Division, for technical assistance and to Ms. M. Suzuki, Pathology Division, for secretarial work. They also thank Mr. S. Tamura, Photocenter, for his photographic work.

References

1 Shimosato Y, Mukai K. Tumors of the mediastinum. In: Rosai J, Sobin LH, eds. Atlas of tumor pathology, 3rd series, fascicle 21. Washington, DC: The Armed Forces Institute of Pathology, 1997; 40–120

2 Suster S, Rosai J. Thymic carcinoma: a clinicopathologic study of 60 cases. Cancer 1991; 15:67:1025–1032

3 Hsu CP, Chen CY, Chen CL, et al. Thymic carcinoma: ten years' experience in twenty patients. J Thorac Cardiovasc Surg 1994; 107:615–620

4 Lucchi M, Mussi A, Basolo F, et al. The multimodality treatment of thymic carcinoma. Eur J Cardiothorac Surg 2001; 19:566–569

5 Berezowski K, Grimes MM, Gal A, et al. CD5 immunoreactivity of epithelial cells in thymic carcinoma and CASTLE using paraffin-embedded tissue. Am J Clin Pathol 1996; 106:483–486

6 Hishima T, Fukayama M, Fujisawa M, et al. CD5 expression in thymic carcinoma. Am J Pathol 1994; 145:268–275

7 Dorfman DM, Shahsafaei A, Chan JK. Thymic carcinomas, but not thymomas and carcinomas of other sites, show CD5 immunoreactivity. Am J Surg Pathol 1997; 21:936–940

8 Yarden Y, Kuang WJ, Yang-Feng T, et al. Human proto-oncogene c-kit: a new cell surface receptor tyrosine kinase for an unidentified ligand. EMBO J 1987; 6:3341–3351

9 Natali PG, Nicotra MR, Sures I, et al. Expression of c-kit receptor in normal and transformed human nonlymphoid tissues. Cancer Res 1992; 52:6139–6143

10 Turner AM, Zsebo KM, Martin F, et al. Nonhematopoietic tumor cell lines express stem cell factor and display c-kit receptors. Blood 1992; 80:374–381

11 Tsuura Y, Hiraki H, Watanabe K, et al. Preferential localization of c-kit product in tissue mast cells, basal cells of skin, epithelial cells of breast, small cell lung carcinoma and seminoma/dysgerminoma in human: immunohistochemical study on formalin-fixed, paraffin-embedded tissues. Virchows Arch 1994; 424:135–141

12 Matsuda R, Takahashi T, Nakamura S, et al. Expression of the c-kit protein in human solid tumors and in corresponding fetal and adult normal tissues. Am J Pathol 1993; 142:339–346

13 Sarlomo-Rikala M, Kovatich AJ, Barusevicius A, et al. CD117: a sensitive marker for gastrointestinal stromal tumors that is more specific than CD34. Mod Pathol 1998; 11:728–734

14 Cole SR, Aylett GW, Harvey NL, et al. Increased expression of c-Kit or its ligand Steel Factor is not a common feature of adult acute myeloid leukaemia. Leukemia 1996; 10:288–296

15 Arber DA, Tamayo R, Weiss LM. Paraffin section detection of the c-kit gene product (CD117) in human tissues: value in the diagnosis of mast cell disorders. Hum Pathol 1998; 29:498–504

16 Heinrich MC, Blanke CD, Druker BJ, et al. Inhibition of KIT tyrosine kinase activity: a novel molecular approach to the treatment of KIT-positive malignancies. J Clin Oncol 2002; 20:1692–1703

17 Henley JD, Cummings OW, Loehrer PJ Sr. Tyrosine kinase receptor expression in thymomas. J Cancer Res Clin Oncol 2004; 130:222–224

18 Pan CC, Chen PC, Chiang H. KIT (CD117) is frequently overexpressed in thymic carcinomas but is absent in thymomas. J Pathol 2004; 202:375–381

19 Rosai J. Histological typing of tumors of the thymus: World Health Organization, international histological classification of tumors. 2nd ed. New York: Springer-Verlag, 1999

20 Caceres-Cortes JR, Alvarado-Moreno JA, Waga K, et al. Implication of tyrosine kinase receptor and steel factor in cell density-dependent growth in cervical cancers and leukemias. Cancer Res 2001; 61:6281–6289

21 Kondoh G, Hayasaka N, Li Q, et al. An *in vivo* model for receptor tyrosine kinase autocrine/paracrine activation: autostimulated KIT receptor acts as a tumor promoting factor in papillomavirus-induced tumorigenesis. Oncogene 1995; 10: 341–347

22 Sekido Y, Obata Y, Ueda R, et al. Preferential expression of c-kit protooncogene transcripts in small cell lung cancer. Cancer Res 1991; 51:2416–2419

23 Sekido Y, Takahashi T, Ueda R, et al. Recombinant human stem cell factor mediates chemotaxis of small-cell lung cancer cell lines aberrantly expressing the c-kit protooncogene. Cancer Res 1993; 53:1709–1714

24 Cohen PS, Chan JP, Lipkunskaya M, et al. Expression of stem cell factor and c-kit in human neuroblastoma: the Children's Cancer Group. Blood 1994; 84:3465–3472

25 Hirota S, Isozaki K, Moriyama Y, et al. Gain-of-function mutations of c-kit in human gastrointestinal stromal tumors. Science 1998; 279:577–580

26 Tian Q, Frierson HF Jr, Krystal GW, et al. Activating c-kit gene mutations in human germ cell tumors. Am J Pathol 1999; 154:1643–1647

27 Piao X, Bernstein A. A point mutation in the catalytic domain of c-kit induces growth factor independence, tumorigenicity, and differentiation of mast cells. Blood 1996; 87:3117–3123

28 Butnor KJ, Burchette JL, Sporn TA, et al. The spectrum of Kit (CD117) immunoreactivity in lung and pleural tumors: a study of 96 cases using a single-source antibody with a review of the literature. Arch Pathol Lab Med 2004; 128:538–543