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Short communication

Signet ring cell adenocarcinoma of the lung with an *EML4–ALK* fusion gene mimicking mucinous (colloid) adenocarcinoma: A case report

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ABSTRACT

We herein report a case of signet ring cell adenocarcinoma of the lung with an *EML4–ALK* fusion gene mimicking mucinous (colloid) adenocarcinoma. A 79-year-old female presented with a pulmonary tumor located in the right lower lobe measuring 21 mm in size. A right lower lobectomy was performed. The postoperative pathological examination revealed signet ring cell carcinoma with abundant mucin pools, and a multiplex RT-PCR analysis revealed the variant 2 inversion of the *EML4–ALK* gene.

1. Introduction

Primary lung adenocarcinomas in which neoplastic cells float in large mucin pools are unusual, and their exact classification is still controversial. Signet ring cell carcinoma is an unusual subtype of adenocarcinoma of the lung. The most prominent pathological feature of this cancer is the intracellular mucin accumulation with a signet ring conformation [1]. Meanwhile, primary mucinous (colloid) adenocarcinoma (MC) is an extremely rare subtype of pulmonary adenocarcinoma that accounts for 0.24% of all lung cancers [2]. MC is described as a variant of adenocarcinoma by the latest edition of the world health organization (WHO) classification of lung cancers and was recently proposed to be colloid adenocarcinoma [1,3]. MC is pathologically characterized by a lesion with dissecting pools of mucin containing islands of neoplastic epithelium [1].

Recently, the echinoderm microtubule-associated protein-like 4 (*EML-4*)–anaplastic lymphoma kinase (*ALK*) gene inversion was detected in 6.7% of Japanese non-small cell lung cancer (NSCLC) patients [4]. The fusion gene encodes a constitutively active oncoprotein with an activated *ALK* kinase, resulting in the aberrant activation of downstream signaling targets including Akt, signal transducer and activator of transcription (STAT) 3, and Ras-extracellular signal regulated kinase (ERK) 1/2 [5]. Several series have reported the clinicopathological factors in patients with the *EML4–ALK* inversion [6,7]. *EML4–ALK* positive lung adenocarcinomas tended to be characterized by a less-differentiated grade, predominantly the acinar subtype or the signet-ring cell subtype in histology[6,7].

We herein report a case of primary signet ring cell adenocarcinoma mimicking MC with the inversion of the *EML4–ALK* gene.

2. Case report

A 79-year-old female who was a never smoker, was referred to our hospital because of left chest pain. Chest computed tomography (CT) performed as an initial screening showed an irregularly formed and well-defined nodule measuring 21 mm in the right lower lobe. The tumor was uniformly enhanced with regard to the density of the mediastinum (Fig. 1). Positron emission tomography (PET) with ^{18}F-fluorodeoxyglucose (FDG) showed positive activity in this lesion (maximum standardized uptake values (SUV_{max}); 4.78) and a hilar lymph node (SUV_{max}; 3.59). No metastatic tumor was detected by brain magnetic resonance imaging (MRI). The results of clinical examination and routine laboratory tests were within normal lim-

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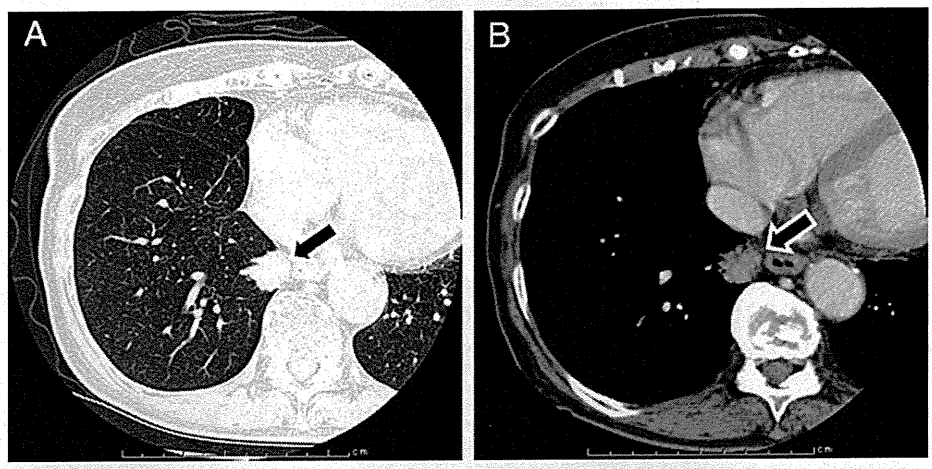

Fig. 1. CT demonstrated a round and well-defined nodule with spiculation measuring 21 mm in diameter in the right lower lobe (arrow). (A) The density of the lung. (B) The density of the mediastinum.

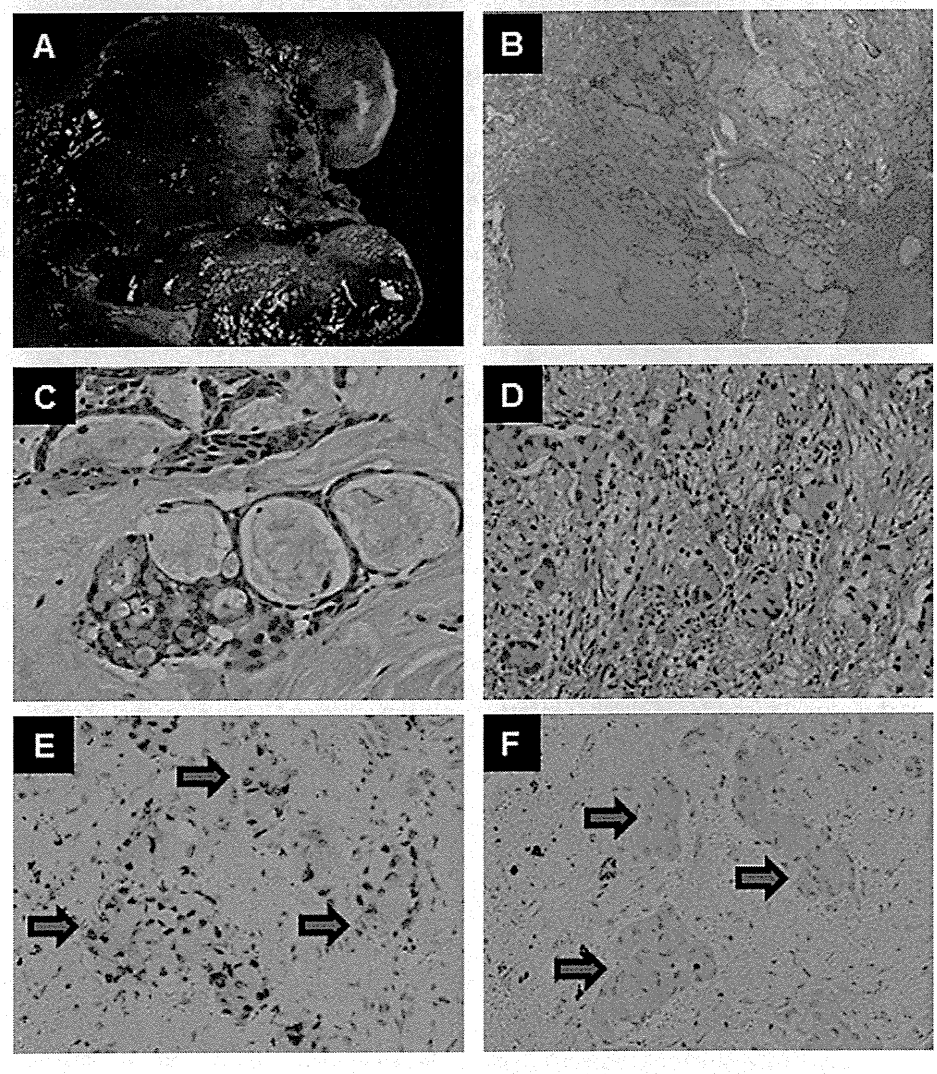

Fig. 2. (A) The cut surface of the tumor shows the nodule is well demarcated and filled with a yellowish-white gelatinous substance. (B) Two-thirds of the tumor was composed of abundant mucin pools that distended the alveoli. (H&E, objective lens magnification; 1.25×). (C and D) Foci of adenocarcinoma (H&E, objective lens magnification; 20×). (E) The nuclei and cytoplasm of the signet ring tumor cells in the fibrous stroma were positive for TTF-1 (arrows) (objective lens magnification; 20×). (F) The cytoplasm of the signet ring tumor cells was weakly positive for CEA (arrows) (objective lens magnification; 20×).

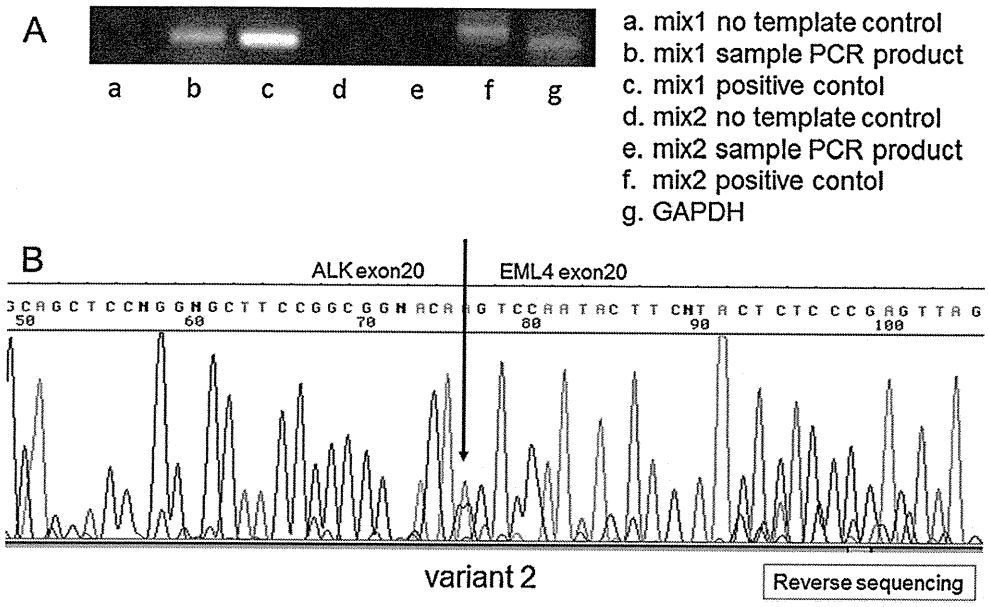

Fig. 3. Genetic analyses. (A) Multiplex RT-PCR to capture all in-frame fusions between *EML-4* and *ALK* messages was conducted with the primers. Mix1 contains variants 1, 2, 4, 7, and KIF5B. Mix2 contains variants 3, 5, and 6. (B) The transition was confirmed to be the *EML4–ALK* inversion-variant 2 by a direct sequencing method.

its except for an elevated serum level of carcinoembryonic antigen (CEA) (15.6 ng/ml).

Because of the elevated level of CEA and the results of the radiological examination, the lesion was clinically suspected to be a primary lung cancer. The clinical TNM classification was T1bN1M0; cStage IIA. Intraoperative aspiration cytology for the primary tumor revealed carcinoma, so a right lower lobectomy and lymph node dissection were performed.

Macroscopically, the well-demarcated tumor contained a yellowish gelatinous substance, thus indicating the presence of an abundant amount of mucin in the tumor (Fig. 2A). Histopathologically, two-thirds of the tumor consisted of mucin pools that distended the alveoli and floating foci of mucinous epithelia, some of which resembled signet ring cells (Fig. 2B and C). The rest of the tumor was composed of acinar adenocarcinoma and signet ring adenocarcinoma with fibrous stroma (Fig. 2D). Columnar mucinous epithelial cells were not evident in this tumor. Immunohistochemically, the tumor cells were positive for CEA, cytokeratin 7 (CK7), and thyroid transcription factor-1 (TTF-1), whereas they were negative for cytokeratin 20 (CK20), MUC5AC, cluster of differentiation 10 (CD10), and CDX-2 (Figs. 2E and F). No tumor cells were detected in the dissected lymph nodes. As a result, the tumor was diagnosed to be a primary signet ring cell adenocarcinoma, and the pathological TNM classification was T1bN0N0; stage IA.

The tumor was examined for inversion of the *EML4-ALK* gene by multiplex RT-PCR analysis of the frozen section, and the result was confirmed by direct sequencing. The sequences of these primers were modified following the previous report [8]. This case was positive for variant 2 of the *EML4-ALK* gene (Fig. 3). The tumor was negative for mutations of the epidermal growth factor receptor (EGFR).

There were no postoperative complications. The patient was discharged on the 8th postoperative day and is doing well at 10 months after the surgery.

3. Discussion

This case was strongly suggested to be MC macroscopically, because the cut surface was yellowish and gelatinous, like MC. Histologically, our case showed the presence of abundant mucin pools, floating foci of the tumor cells, and expansion of the alveolar spaces. These findings seemed to be compatible with the histological features of MC [2]. However, a well differentiated columnar mucinous epithelium could not be detected in the tumor. On the contrary, foci of signet ring cell adenocarcinoma were recognized in the fibrous stroma, accompanied by floating signet ring cells in the mucin pools. Therefore, the tumor was finally diagnosed to be primary signet ring cell carcinoma mimicking MC.

Rossi et al. divided MC into the two distinct types: the classic goblet cell-type and the signet-ring cell type [2]. The classic goblet cell-type is more frequent and consists of prominent pools of mucin-disrupting alveoli that invade the adjacent lung. Columnar mucinous neoplastic elements float into the mucus and line the alveolar structures. The signet-ring cell type is rare, and shows rich mucin pools expanding the alveolar spaces. Neoplastic cells floating in mucin pools have a signet ring cells appearance with significant cytologic atypia. Histologically, our case is considered to mimic the pattern of the signet ring cell cancer. The signet ring cell type of MC shows positive immunostaining for TTF-1, CK-7, and MUC-5AC [2]. Our case showed positive immunostaining for TTF-1and CK-7, and negative staining for CDX-2, MUC5AC, CK-20, and MUC2. On the other hand, signet ring cell carcinoma has been reported to demonstrate positive immunostaining for TTF-1 and CK-7, while it is negative for MUC5 [9]. Therefore, the immunohistochemical features indicate signet ring cell carcinoma rather than MC. Immunohistochemical findings might therefore play an important role in distinguishing signet ring cell carcinoma from the signet ring cell type of MC.

Activating mutations within the *EGFR* have been identified in lung cancers, and chemical inhibitors of the kinase activity of EGFR have been found to be effective in the treatment of a subset of lung cancer patients harboring such mutations [10–12]. Recently, tumors with the *EML4–ALK* gene fusion have also been receiving attention because they may be the target of new molecular targeting therapy. In fact, *EML4–ALK*-dependent cells undergo apoptosis when treated with an *ALK*-inhibitor [4,13].

A variety of histological features reported to be associated with *EML4–ALK* gene fusion positive lung cancers were reported in two

articles [7,14]. Inamura et al. reported that the acinar pattern was mostly associated with *EML4–ALK* gene fusion positive lung adenocarcinomas in an Asian population [7]. *EML4–ALK* gene fusion positive lung adenocarcinomas comprised 11 (4%) of 253 patients in their series [7]. According to the predominant subtypes of adenocarcinomas, 6 of 11 *EML4–ALK* gene fusion positive lung cancers (55%) were subclassfied as acinar adenocarcinomas, and the other 5 cancers (45%) were subclassfied as papillary adenocarcinomas. On the other hand, Rodig et al. reported that the solid pattern and the signet-ring cell histology were most commonly associated with this gene fusion in Western patients [14]. The *EML4–ALK* gene fusion positive lung adenocarcinomas were found on 16 (5%) of their 326 patients. With regard to the predominant subtypes of adenocarcinomas, 11 of the 16 *EML4–ALK* gene fusion positive lung cancers (69%) in this study were subclassfied as solid adenocarcinomas, while the other 4 cancers (25%) were subclassfied as acinar adenocarcinomas, and the other tumor was subclassfied as bronchioloalveolar carcinoma. Of these 16 tumors, 12 (75%) had the signet ring cells. Similarly, Shaw et al. proposed that there is a close relationship between the signet ring cell pattern and *EML4–ALK* [6]. The present report was subclassfied as a signet ring adenocarcinoma mimicking MC with the *EML4–ALK* gene fusion. On the other hand, no report has yet diagnosed an MC with the *EML4–ALK* gene fusion. In the future, the further analysis of the expression of such genes as *EML4–ALK* and *EGFR* is strongly recommended as an important part of the histopathological classification of lung malignancies.

4. Conclusions

This report describes a case of *EML4–ALK*-positive signet ring cell adenocarcinoma of the lung mimicking MC.

Conflict of interest statement

All authors have declared that they have no conflict of interest.

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SURGERY TODAY

Original Article

Concurrent Chemoradiotherapy Using Cisplatin Plus S-1, an Oral Fluoropyrimidine, Followed by Surgery for Selected Patients with Stage III Non-Small Cell Lung Cancer: A Single-Center Feasibility Study

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Abstract

Purpose. This single-institutional study was designed to determine whether S-1, an oral fluoropyrimidine, plus cisplatin with concurrent radiotherapy is feasible as an induction treatment for locally advanced non-small cell lung cancer (NSCLC).

Methods. Eighteen patients were analyzed in this study from July 2005 to March 2008. The patients received 40 mg/m^2 S-1 orally twice per day on days 1 through 14 and 22 through 35, and cisplatin (60 mg/m^2) was injected intravenously on days 8 and 29. The patients also underwent radiotherapy, and received a total dose of 40 Gy in 20 fractions beginning on day 1. Surgical resection was performed from 3 to 6 weeks after completing the induction treatment.

Results. Nine (50%) of the 18 patients who received the induction treatment achieved a partial response. One patient refused to undergo surgery. The remaining 17 patients underwent a complete surgical resection. There were no deaths nor any major morbidities during the perioperative period. The recurrence-free survival and overall survival rate at 2 years for the patients who underwent resection were 63.3% and 88.2%, respectively.

Conclusion. Induction treatment using S-1 plus cisplatin and concurrent radiotherapy and surgical resection for selected patients with stage III NSCLC is a feasible and promising new treatment modality.

Key words Non-small cell lung cancer · Stage III · Induction chemoradiotherapy · S-1 · Cisplatin · Surgical resection

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Introduction

The standard treatment for unresectable stage III non-small cell lung cancer (NSCLC) is concurrent chemoradiotherapy. Two large multicenter randomized phase III trials have explored the role of surgery versus radiotherapy after induction treatment for stage III-N2 NSCLC.[1,2] No data have so far shown that neoadjuvant treatment followed by surgery results in prolonged survival in comparison with adequate chemoradiation alone. Surgery after chemo-radiotherapy therefore remains controversial for patients with this stage of disease.

Both cisplatin and 5-fluorouracil (5-FU) have a radiosensitizing effect for NSCLC.[3,4] UFT contains tegafur (a prodrug that is converted to 5-FU by cells) and uracil at a 1:4 molar ratio concentration. Ichinose et al. reported the results of a multi-institutional phase II trial, which demonstrated that UFT plus cisplatin with concurrent radiotherapy is a feasible and effective treatment for locally advanced NSCLC. The response rate and median survival of locally advanced unresectable stage III (IIIA 20%, IIIB 80%) patients in that study were 81% and 16.5 months, respectively, with low-grade toxicities.[5] Ichinose et al. also reported a phase II trial using UFT plus cisplatin with concurrent radiotherapy as induction treatment for marginally resectable stage IIIB NSCLC. The surgical morbidity and mortality rates were 36% and 4%, respectively. The calculated 1- and 3-year survival rates in the resected patients were 82% (95% confidence interval [CI]: 66%–98%) and 67% (95% CI 47%–87%), respectively.[6] S-1 (Taiho Pharmaceutical, Tokyo, Japan) is an orally active combination of tegafur, gimeracil (an inhibitor of dihydropyrimidine dehydrogenase, which degrades fluorouracil), and oteracil (which inhibits the phosphorylation of fluorouracil in the gastrointestinal tract, thereby reducing the

gastrointestinal toxicity of fluorouracil) in a molar ratio of 1:0.4:1.[7] S-1 was developed to improve the tumor-selective cytotoxicity of 5-FU, while reducing gastrointestinal toxicity through the addition of these modulators. Ohyanagi et al. reported a phase II trial, which demonstrated that S-1 plus cisplatin with concurrent radiotherapy was a promising treatment for unresectable stage III NSCLC. The response rate and median survival were 87.5% and 33.1 months, respectively, with mild toxicities. The 1- and 2-year survival rates were 89.5% and 56%, respectively.[8]

We designed a single-institutional feasibility study of the combination chemotherapy using S-1 plus cisplatin with concurrent radiotherapy followed by surgical resection for selected patients with stage III NSCLC.

Patients and Methods

Patients

Eighteen patients were registered from July 2005 to March 2008. All patients provided their written informed consent according to institutional guidelines. All patients were required to be at least 20 years of age, with an Eastern Cooperative Oncology Group (ECOG) performance status (PS) of 0 to 1, with no uncontrolled cardiac or hepatic disease. All had adequate bone marrow function (defined as a total leukocyte count $\geq 3.5 \times 10^9$/l, absolute neutrophil count [ANC] $\geq 2 \times 10^9$/l, platelet count $\geq 100 \times 10^9$/l, and hemoglobin level ≥9.0 g/dl); adequate renal function (defined as a serum creatinine level <1.5 mg/dl); adequate hepatic function (defined as a total bilirubin level <1.5 mg/dl and serum aspartate aminotransferase and/or alanine aminotransferase levels <2 times the upper normal limit for the laboratory); no severe cardiac disease or arrhythmia on an electrocardiogram; and a room air oxygen partial pressure >60 mmHg. The clinical or pathological stage of the disease was based on the General Rules for Clinical and Pathological Records of Lung Cancer (6th edition) edited by the Japan Lung Cancer Society.[9] A contralateral mediastinal lymph node was defined as being metastatic when both the ipsilateral and contralateral mediastinal lymph nodes were swollen, with a short diameter of more than 1.5 cm determined by high-resolution thin-section computed tomography (CT) scans.

Mediastinoscopy was not routinely performed, although it is sometimes considered a gold-standard criterion for the evaluation of N2 disease. In addition, the presence of a region of focal low density (other than fat) suggesting necrosis, or surrounding fat infiltration suggesting extrafascial extension, were used as malignant criteria for the evaluation of mediastinal lymph nodes on CT. Most of the cases were suspected to have invasive and extranodal expansion. The disease was defined to be potentially and technically resectable if a tumor appeared to be removable by the replacement of the superior vena cava or great vessels, carinal resection, a partial resection of vertebrae, a resection of the involved bilateral mediastinal or supraclavicular lymph nodes, and that concurrent chemoradiotherapy was preferable to a definitive resection to render their disease resectable. The histological analysis of the tumor was based on the World Health Organization classification for cell types.[10] The clinicopathological characteristics of the patients are shown in Table 1. An "unclassified carcinoma" indicates a malignant epithelial neoplasm that cannot be placed into one of the categories.

Table 1. Clinicopathological characteristics of the patients

Parameter	No.
Age (years), median (range)	59 (47–77)
Sex	
Male	15
Female	3
Performance status (ECOG)	
0	12
1	6
Histological type	
Adenocarcinoma	9
Squamous cell carcinoma	5
Large cell carcinoma	1
Pulmonary blastoma	1
Unclassified carcinoma	2
Clinical stage	
IIIA	10
IIIB	8

ECOG, Eastern Cooperative Oncology Group

Treatment Schedule

S-1 was administered orally at 40 mg/m^2 twice per day on days 1 through 14 and 22 through 35. The actual dose of S-1 was 40 mg twice a day in a patient with a body surface area (BSA) <1.25 m^2, 50 mg twice a day for those with a BSA of 1.25 m^2 but <1.5 m^2, and 60 mg twice a day for those with a BSA >1.5 m^2. Cisplatin (60 mg/m^2) was administered during a 90-min infusion on days 8 and 29 when patients were hydrated with at least 2500 ml of saline infusion. Radiotherapy was administered in five fractions per week from a megavolt linear accelerator at a daily dose of 2 Gy from day 1 up to a total dose of 40 Gy (20 fractions). The target volume included the primary disease site with a 2-cm margin around the mass, and the ipsilateral hilum. The entire width of the mediastinum was included with a 2-cm margin around the radiographically visible area of involvement (as determined by a pretreatment CT scan). The inferior margin extended 4 cm below the carina or 2 cm below the radiographically visible tumor mass. The supraclavicular fossa was not

irradiated when no tumor was detected in that tissue by either a physical or radiographic examination. The blood cell counts and chemistries were examined at least once a week. Patients were not to receive prophylactic granulocyte-colony stimulating factor (G-CSF) during any cycle. The use of G-CSF was allowed only for patients who had an ANC $<0.5 \times 10^9$/l, neutropenic fever, or documented infections while neutropenic.

Evaluations

The response was evaluated according the RECIST (Response Evaluation Criteria in Solid Tumors),[11] and toxicity criteria were based on the Common Terminology Criteria for Adverse Events (CTCAE), version 3.0.

Statistical Analysis

The duration of the recurrence-free survival (RFS) was calculated from the starting date of induction chemoradiotherapy until either the first evidence of recurrence or death due to any cause, or until the last follow-up (censored). The overall survival (OS) was calculated from the starting date of induction chemoradiotherapy until death due to any cause, or the last follow-up (censored). The survival curve was made using the Kaplan–Meier method.[12] All data were analyzed using the IBM SPSS Statistics 18 software package (SPSS Japan, an IBM company, Tokyo, Japan).

Results

Induction Treatment and Toxicity

All patients received the planned dose of radiotherapy, and 15 patients (83%) underwent two cycles of chemotherapy as induction treatment. Nine (50%) of the 18 patients achieved a partial response after receiving the induction treatment (95% CI, 21%–72%). The hematological and nonhematological toxicities of grade 2 or worse that were observed during the induction chemoradiotherapy are listed in Table 2. Leukopenia was the most frequently observed adverse event; however, the incidence of grade 3 or 4 was only 22.2%. The incidence of the other adverse events of grade 3 or 4 toxicities was 11.1% for neutropenia and anemia. This regimen was therefore associated with manageable toxicity. No toxic deaths occurred. Severe nonhematological toxicity was uncommon.

Surgery

One patient refused to undergo surgery. The remaining 17 patients underwent a complete surgical resection (complex pneumonectomy in four patients including 3 intrapericardial pneumonectomies, 3 simple pneumonectomies, and 6 complex lobectomies including 3 sleeve lobectomies and 4 simple lobectomies). There were no deaths or any major morbidity during the perioperative period other than a rethoracotomy, which was performed for bleeding or chylothorax in one patient each.

Table 2. Hematological and nonhematological toxicities observed during induction chemoradiotherapy

	No. of patients (%)		
	Grade 2	Grade 3	Grade 4
WBC	7 (39)	4 (22)	0
ANC	8 (44)	2 (11)	0
Hb	1 (6)	2 (11)	0
Plt	0	0	0
Febrile neutropenia	0	1 (6)	0
AST	1 (6)	0	0
ALT	0	0	0
Hyperbilirubinemia	0	0	0
Hypocalcemia	1 (6)	0	0
Hyperkalemia	1 (6)	0	0
Fatigue	1 (6)	0	0
Nausea/vomiting	1 (6)	0	0
Mucositis	1 (6)	0	0
Dermatitis	3 (17)	0	0
Esophagitis	1 (6)	0	0

WBC, white blood cell count; ANC, absolute neutrophil count; Hb, hemoglobin; Plt, platelets; AST, aspartate aminotransferase; ALT, alanine aminotransferase

Pathological Findings

There were three pathological complete responses (Ef3: no viable tumor cells in resected specimens), and some pathological response was recognized in all of the remaining patients (Ef1a: ≥2/3 viable tumor cells, in 3 patients; Ef1b: 1/3 ≤ viable tumor cells < 2/3, in 2 patients, Ef2: <1/3 viable tumor cells, in 9 patients).

Survival and Recurrence

The median observation time was 29 months. The calculated RFS (Fig. 1) and OS (Fig. 2) rates at 2 years for resected patients were 63.3% and 88.2%, respectively. Recurrence developed in 6 of the 17 resected patients. The recurrence site was distant in 5 patients (brain in 2 patients, contralateral lung in 2, and ipsilateral neck lymph nodes in 1), and 1 patient had a local recurrence in the ipsilateral mediastinal lymph nodes.

Discussion

S-1 plus cisplatin with concurrent radiotherapy followed by surgery for selected stage III NSCLC is feasible and

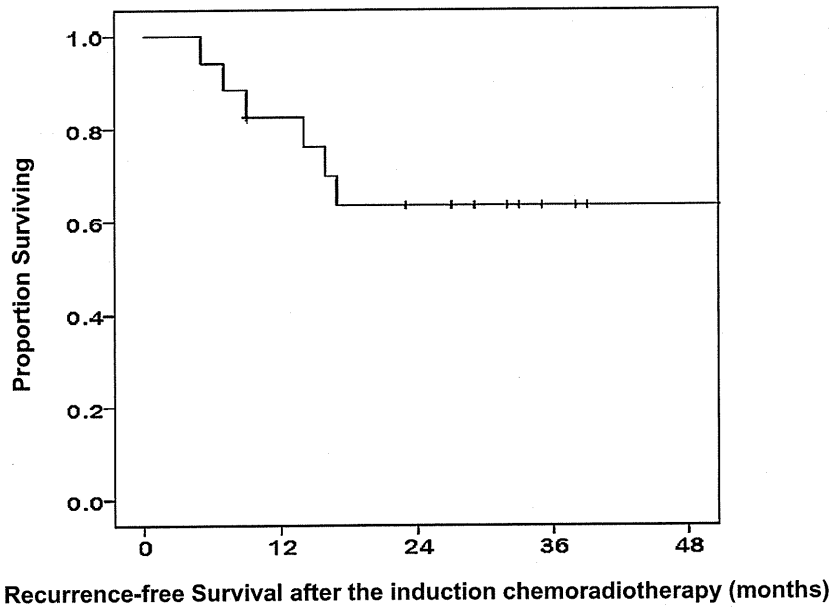

Fig. 1. Recurrence-free survival curve of patients who underwent a resection after receiving induction chemoradiotherapy

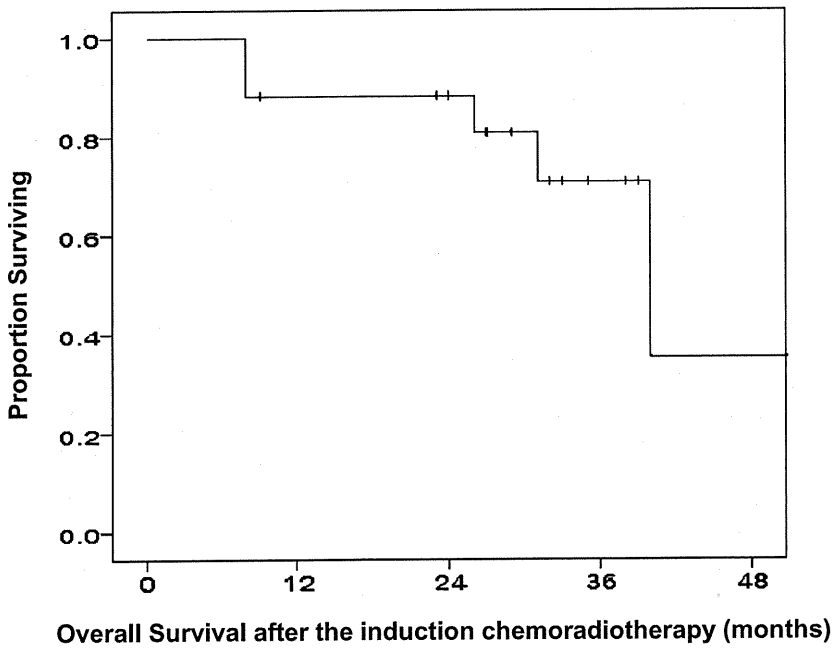

Fig. 2. Overall survival curve of patients who underwent a resection after receiving induction chemoradiotherapy

appears to be promising. A previous study using UFT plus cisplatin with concurrent radiotherapy as induction treatment for selected patients with stage IIIB NSCLC indicated that the 1- and 3-year survival rates in all patients were 73% and 56%, respectively.[6] The present study exceeds those results, without any severe toxicities. However, the response rate was 50% in the present study, which seems relatively low compared with other studies. Nevertheless the upper CI limit was over 70% in this study, so it would not necessarily be low if a larger number of patients were to be studied. A multi-institutional phase II trial must be conducted to establish the effectiveness of this induction treatment.

Surgery after chemoradiotherapy remains controversial for patients with stage IIIA-N2 NSCLC. Recently, Albain et al.[1] and van Meerbeeck et al.[2] reported the definitive data of two trials comparing chemotherapy and radiotherapy followed by surgical resection with

chemotherapy and radiotherapy for stage IIIA-N2 NSCLC. The first trial (INT 0139) used concurrent chemoradiotherapy as induction treatment. Four hundred and twenty-nine patients were randomly assigned at a 1:1 ratio to concurrent induction chemotherapy with cisplatin and etoposide plus radiotherapy (45 Gy). Patients in group 1 underwent a resection if there was no progression and those in group 2 continued radiotherapy uninterrupted up to 61 Gy. Two additional cycles of cisplatin and etoposide were given in both groups. The progression-free survival (PFS) in group 1 was better than in group 2, with a median of 12.8 versus 10.5 months (hazard ratio [HR] = 0.77, P = 0.017). However, the median OS was 23.6 months in group 1 versus 22.2 months in group 2 (HR = 0.87, P = 0.24).[1]

The latter study (EORTC 08941) was a randomized trial of radical surgery versus radiotherapy after a response to three cycles of platinum-based induction chemotherapy. Five hundred and eighty-two patients were registered in this study. The median and 5-year OS for patients randomly assigned to resection versus radiotherapy were 16.4 vs 17.5 months and 15.7% vs 14%, respectively (HR = 1.06, P = 0.6). The rates of PFS were also similar in both groups. Surgery therefore improved neither PFS nor OS in comparison with radiotherapy.[2]

Of interest, the INT 0139 trial found that deaths by the type of surgery were 1/98 (1%) for lobectomies and 14/54 (26%) for pneumonectomies, respectively.[1] An exploratory analysis determined that the median survival was improved for patients who underwent a lobectomy (33.6 vs 21.7 months, P = 0.002), but not for those who underwent a pneumonectomy (18.9 vs 29.4 months), versus chemotherapy plus radiotherapy without surgery. The investigators hypothesized that trimodality treatment could be beneficial if a complete resection with lobectomy can be done after chemotherapy plus radiotherapy, or if the mortality rate of pneumonectomy can be decreased.[1] An unplanned subgroup analysis of EORTC 08941 also found that patients who underwent a lobectomy or bilobectomy had better outcomes than those who had a pneumonectomy (5-year survival 27% vs 12%, respectively; P = 0.009).[2] A randomized phase III trial by the German Lung Cancer Cooperative group compared the intensity of the induction regimen: neoadjuvant concurrent chemoradiation versus chemotherapy alone.[13] Surgery was followed by radiotherapy in the latter group. The severe toxicity and mortality rates were significantly higher in the group of patients with preoperative chemoradiation, with almost double the postoperative mortality rate (9% vs 4.5%), especially after pneumonectomy. These three studies suggest that a pneumonectomy should therefore be avoided after induction chemoradiotherapy. However, Allen et al. reported that a pneumonectomy after chemoradiation could be safely accomplished in patients with advanced NSCLC in a single-institutional series. The 30- and 100-day mortality rates in their study were 6% and 10%, respectively. Their median survival was 23 months, with a 2-year survival rate of 49%.[14] There were neither deaths nor any major morbidities during the perioperative period in the present study, despite the fact that four complex pneumonectomies and three simple pneumonectomies were performed, confirming the findings by Allen et al.

Endobronchial ultrasound (EBUS)-guided transbronchial needle aspiration is emerging as an alternative to mediastinoscopy for mediastinal lymph node evaluation in NSCLC. However, for routine staging of the upper mediastinum in NSCLC, the benefits of EBUS over mediastinoscopy remain unproven. Therefore, mediastinoscopy is still the gold standard for mediastinal lymph node staging of NSCLC.[15] However, the procedure requires general anesthesia, and complications can occur. The new American College of Chest Physicians evidence-based practical guidelines for invasive staging of lung cancer were recently published.[16] Accordingly, invasive staging is not recommended for patients with extensive mediastinal infiltration (grade of recommendation: 2C). However, staging by CT or positron emission tomography scanning is still not sufficiently accurate, and invasive confirmation of the radiographic stage is recommended for patients with discrete node enlargement (grade of recommendation: 1B).

In conclusion, chemotherapy using S-1 plus cisplatin and concurrent radiotherapy as induction treatment followed by a surgical resection for selected patients with stage III NSCLC is feasible and appears to be a promising new treatment modality. A multi-institutional phase II trial is now being planned to establish the effectiveness of this induction treatment for patients with locally advanced NSCLC.

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LKB1 Mutations Frequently Detected in Mucinous Bronchioloalveolar Carcinoma

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Objective: *LKB1* mutations are common in patients with Peutz–Jeghers syndrome, which is characterized by mucocutaneous pigmentation, intestinal polyps and a high incidence of cancers at variable sites. This study investigated the status of the *LKB1* gene in mucinous bronchioloalveolar carcinoma with or without Peutz–Jeghers syndrome.
Methods: Three mucinous bronchioloalveolar carcinoma tumors from two Peutz–Jeghers syndrome patients and seven tumors from sporadic mucinous bronchioloalveolar carcinoma patients were collected by surgery between 2002 and 2008, and high molecular weight genomic DNA was extracted from them. The nucleotide sequences in exons 1–9 of *LKB1* were determined by genomic polymerase chain reaction-direct sequencing. The loss of heterozygosity was analyzed by high-resolution fluorescent microsatellite analysis using two microsatellite markers that encompass the *LKB1* locus, D19S886 and D19S565. The mutations of *KRAS*, *EGFR* and *p53* were also evaluated.
Results: The germline mutation of *LKB1* in the Peutz–Jeghers syndrome patients was identified as G215D by analyzing genomic DNA from normal lung tissue specimens. Furthermore, two of the three mucinous bronchioloalveolar carcinomas from these Peutz–Jeghers syndrome patients exhibited additional somatic mutations. On the other hand, four of seven sporadic 'non-Peutz–Jeghers syndrome' mucinous bronchioloalveolar carcinomas had *LKB1* mutations. Loss of heterozygosity analyses revealed allelic loss in two tumors with *LKB1* mutations. As a result, 70% of the mucinous bronchioloalveolar carcinomas exhibited *LKB1* mutations. *KRAS*, *EGFR* and *p53* mutations were mutually exclusive and observed in four, two and one tumors, respectively. Among them, five mutations occurred concomitantly with *LKB1* mutations.
Conclusions: The relatively high frequency of *LKB1* mutations in mucinous bronchioloalveolar carcinoma patients may therefore suggest its involvement in lung carcinogenesis, at least in mucinous bronchioloalveolar carcinoma.

Key words: tumor suppressor gene – hereditary disease – bronchioloalveolar carcinoma – loss of heterozygosity

INTRODUCTION

Liver kinase B1 (*LKB1*) is a gene encoding a serine–threonine kinase, which was initially deposited in the database by Nezu et al. in 1996, without writing a published report, in a screen aimed at identifying new kinases. In 1997, Hemminki et al. (1) revealed that the locus responsible for Peutz–Jeghers syndrome (PJS) was located on chromosome 19p13.3, where they then found the locus encoding *LKB1* with diverse mutations in PJS families (2). Another group also reported mutations in the *LKB1* gene

Table 1. *LKB1* mutations in mBACs

Tumor	Age	Sex	PYI	Size (mm)	LOH at D19S886	*LKB1* mutation									LOH at D19S565	*KRAS*	*EGFR*	*p53*
						EX01	02	03	04	05	06	07	08	09				
PJS																		
1	68	F	0	82	NI					E223L	G251D[a]				−	G12C		
2	43	F	0	29	NI	Y60X					G251D[a]				−	G12V		
3[b]	44	F	0	10	NI						G251D[a]				−			
Non-PJS																		
4	65	F	20	43	+								F354L		+		Del EX19	
5	69	M	42	90	NI				D194H						+			Y220C
6	55	F	44	59	−	D53T-63X									NI		Del EX19	
7	79	F	0	8	NI								F354L		NI			
8	61	M	82	10	−										−	G12D		
9	76	M	47	15	−										−	G12D		
10	69	M	66	29	NI										NI			

LKB1, liver kinase B1; mBACs, mucinous bronchioloalveolar carcinoma; PYI, pack-year index; LOH, loss of heterozygosity; PJS, Peutz–Jeghers syndrome; NI, not informative.
[a]Identical base substitution mutation confirmed in the germline.
[b]Tumor 3 is a secondary carcinoma that occurred in the PJS patients with tumor 2.

and called this enzyme serine–threonine kinase 11 (*STK11*) (3).

PJS is a rare autosomal-dominant disease characterized by mucocutaneous pigmentation and gastrointestinal hamartomatous polyposis (4,5). Furthermore, patients with PJS have an increased cancer risk, especially for cancers of gastrointestinal origin (6). Tumors arising from PJS are characterized by mucinous phenotypes, as are often observed in gastrointestinal tumors [i.e. adenoma malignum in the cervix (7), pancreatic adenocarcinoma (8) and intraductal pancreatic mucinous neoplasms (9)]. *LKB1* mutations are not as common in sporadic cancers except for non-small cell lung cancers (NSCLC) (10), about a third of which exhibit *LKB1* mutations (11–13). The representative mucinous NSCLC is mucinous bronchioloalveolar carcinoma (mBAC). mBAC is relatively rare among NSCLC, and there is only one case report of a PJS patient with mBAC (14).

The *LKB1* mutations in two PJS patients with mBAC were herein evaluated, along with sporadic cases of mBAC.

PATIENTS AND METHODS

Patients

Seventeen patients with mBAC were identified from 512 consecutive Japanese lung adenocarcinomas that underwent surgery at the Department of Thoracic Oncology, National Kyushu Cancer Center, from 2002 to 2008. Frozen tissue specimens were collected from 10 mBACs out of nine patients (four male and five female; age range: 43–79 years; median: 68 years; Table 1): 3 mBACs were derived from two PJS patients, who belonged to the same family. Tumor 1 was from a woman and Tumors 2 and 3 were from her daughter. Tumors 4–10 were from sporadic mBAC patients. Written informed consent was obtained from all patients, and ethical approval was obtained from the institutional review board of the National Kyushu Cancer Center. The histology of mBAC was determined based on hematoxylin and eosin staining according to the criteria of the World Health Organization (15). High-molecular-weight genomic DNA was extracted from the surgically resected specimens by standard phenol–chloroform methods and then was stocked in the bio-bank at our institute.

Mutation and Loss of Heterozygosity Analysis of the *LKB1* Gene

PCR primers were designed to cover all of the exons in the *LKB1* gene (Table 2). Twenty-five nanograms of genomic DNA from the bio-bank were used for each PCR amplification. The sequences of the primers are listed in Table 2. Direct sequencing of the PCR products was performed using the ABI Prism 310 Genetic Analyzer (Perkin-Elmer). All sequencing reactions were performed in both forward and reverse directions. Mutations were confirmed by an analysis of at least two independent PCR amplifications.

Loss of heterozygosity (LOH) was analyzed by a high-resolution fluorescent microsatellite analysis (HRFMA)

using two microsatellite markers (D19S886 and D19S565) that encompass the *LKB1* locus. Specific primers were designed for these microsatellite markers (Table 2). Separation was done with a four-color laser-induced fluorescence capillary electrophoresis system (ABI Prism 310 Genetic Analyzer, Perkin-Elmer). The collected data were evaluated using the Genescan analysis software package 310 Genescan v. 3.1.2 (16).

Mutation Analysis of the *EGFR*, *KRAS* and *p53* Genes

Genomic PCR-direct sequencing was performed for exons 18–21 of the epidermal growth factor receptor (*EGFR*) gene, for codons 12–13 of the *KRAS* gene and for exons 5–9 of the *p53* gene (17,18). The detailed sequences of the primers are available on request.

Table 2. Primers used for analyses

LKB1	Primer sequences	
	Forward	Reverse
EX01	5′-aacacaaggaaggaccgctc-3′	5′-aagacagaaccatcagcacc-3′
EX02	5′-aggtacgccacttccacagg-3′	5′-ccattgccacaatggctgac-3′
EX03	5′-ccttttcagaggggtggctgag-3′	5′-tatcaggacaagcagtgtgg-3′
EX04	5′-cctggacttctgtgacttcc-3′	5′-cgaacgggtgcagtgcctgtg-3′
EX05	5′-caccctcaaaatctccgacc-3′	5′-agctgcccaagacgcagagg-3′
EX06	5′-cgtcaaccaccttgactgac-3′	5′-ccaaccctacatttctgcac-3′
EX07	5′-aggagcgtccaggtatcac-3′	5′-ctagcgcccgctcaaccag-3′
EX08	5′-ctgggtcggaaaactggacc-3′	5′-gacgtgggattggccaccag-3′
EX09	5′-ttcaggctggatacacctgg-3′	5′-ggtcaccatgactgactagc-3′
D19S565	5′-tcgaggcagcgtgattgcac-3′	5′-gatcattcctgtagtgatgc-3′
D19S886	5′-cattttactggctggcacttg-3′	5′-gtgttgggaacattcagctc-3′

RESULTS

LKB1 Mutations in PJS Patients

The *LKB1* mutation in PJS was confirmed by analyzing the genomic DNA from normal lung tissue specimens. Both patients, the mother and her daughter, were proven to possess the G251D mutation. The same mutation was observed in the three tumors derived from those patients. In addition, the mother's tumor had E223L, and one of the daughter's tumors had Y60X. Both of these two tumors are thought to have compound heterozygotes. LOH were not informative in these three tumors (Table 1).

LKB1 Mutations in Sporadic mBACs

Four of the seven sporadic mBACs had *LKB1* mutations: F354L in two and D194H and 63-stop in one each. LOH analyses revealed definite allelic loss in Tumor 4 (LOH was positive for both D19S886 and D19S565) and possible allelic loss in Tumor 5 (LOH was positive for D19S565 but not informative for D19S886). These results therefore explain one distinct (Tumor 4) and another possible (Tumor 5) biallelic inactivation of *LKB1* (Fig. 1). The other two sporadic cases exhibited neither LKB1 mutations nor LOHs.

Finally, 70% of mBACs exhibited LKB1 mutations. An analysis of the sporadic cases, excluding the PJS cases, revealed that the percentage was still as high as 57%.

Mutations in *KRAS*, *EGFR* and *p53*

A *KRAS* mutation was observed in four tumors: G12D in two and G12C and G12V in one each. The deletion in exon 19 (del E746-A750) of *EGFR* was observed in two tumors. The *p53* mutation (Y220C) was observed in one tumor. The mutations in *KRAS, EGFR* and *p53* were mutually exclusive (Table 1). Commonly, LKB1 mutations co-existed with

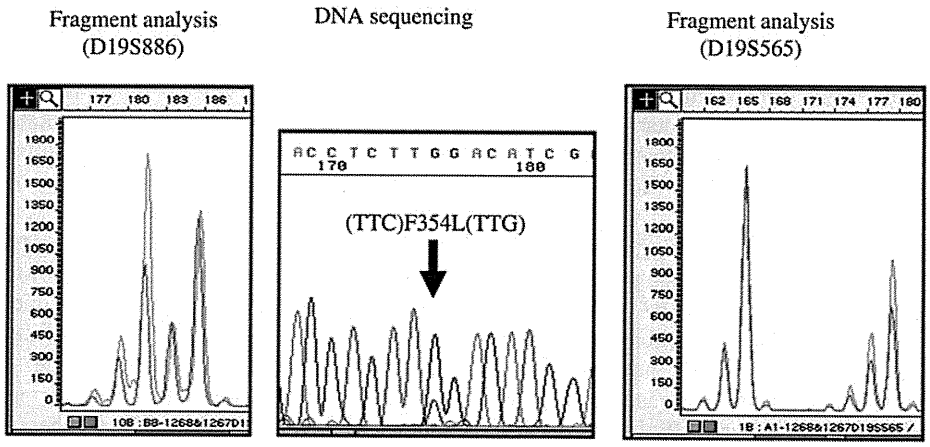

Figure 1. Representative mutation and loss of heterozygosity (LOH) analyses of *LKB1* are shown (Tumor 4). F354L was recognized at the black arrow by DNA sequencing. LOH was determined by fragment analyses using two microsatellite markers that encompassed the *LKB1* locus (D19S886 and D19S565).

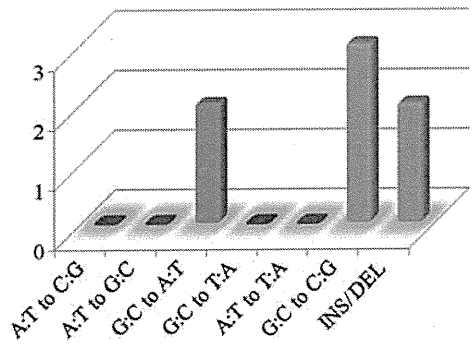

Figure 2. The spectrum of *LKB1* mutations is shown. G:C to A:T were E223L and G251D, and G:C to C:G were D194H and F354L. Y60X was a single-base insertion and D53T-63X was a single-base deletion.

KRAS mutations in Tumors 1 and 2, which are derived from familiar PJS cases; EGFR mutations in Tumors 4 and 6; and p53 mutation in Tumor 5.

Mutation Spectra of *LKB1* in mBAC

The mutation spectra of *LKB1* were analyzed. The germline mutation in the PJS pedigree was the G:C to A:T transition. The somatic mutations identified were one G:C to A:T transition, three G:C to C:G transversions, one single-nucleotide insertion and one single-nucleotide deletion (Fig. 2). None of the other mutations were observed.

The mutation spectra of *KRAS* and *p53* are G:C to T:A in two tumors, G:C to A:T in two tumors and T:A to C:G in one tumor. Both *EGFR* mutations were deletions.

DISCUSSION

The current study demonstrated the frequency of *LKB1* mutation in mBAC to be relatively high (4/7; 57%) in sporadic lung tumors; it was found in all of the tumors derived from PJS patients, although the frequency of *LKB1* mutation is reported to be 4–5% among lung cancers in Japan (19,20). *LKB1* mutations in lung cancer were related with male sex, smoking history and *KRAS* mutations (10,13,19,20). No significance of these parameters was observed in the current series, other than the relationship between *LKB1* mutation and mBAC. The mutations observed here have been reported in PJS patients, which inactivate kinase activity or impair farnesylation at the C-terminus (21,22).

Biallelic inactivation resulted from *LKB1* mutations with LOHs or from compound heterozygotes recognized in PJS cases account for the importance of *LKB1* mutation in mBAC tumorigenesis. *LKB1* is a tumor suppressor gene that causes G1 arrest when overexpressed (23). Further studies have shown that LKB1 acts as a master kinase controlling cellular polarity via MAP/microtubule affinity-regulating kinase, energy metabolism and the mammary target of the rapamycin (mTOR) pathway via AMP-activated protein kinase (AMPK) (24). Enterocytes with wild-type LKB1 differentiate and gain polarity once attached to the basal layer or become confluent; nevertheless, mutant LKB1 cannot gain polarity (25).

LKB1's role in polarity control was also confirmed from *in vivo* experiments by Shorning et al. (26). They also suggested the existence of possible relationships between LKB1 inactivation, polarity deregulation and excessive mucin production. They constructed a conditional knockout mouse model of LKB1. The epithelial cells of the mouse small intestine demonstrated an increased size and number of mucin-containing, goblet-cell-like cells when LKB1 was inactivated in the small intestines of mice by intraperitoneal injection of β-naphthoflavone. These undifferentiated cells showed features that were somewhere intermediate between Paneth and goblet cells (26). They concluded that these phenomena could explain the pathological aspect of polyp development in PJS patients, because alterations in goblet cells and elevated mucin production are commonly observed in hamartomas developing in PJS patients (27). Pathologically, similar features are observed in mBACs. mBAC cells are characterized by goblet cell dysplasia of the lining surface cells in the terminal respiratory unit, with excessive mucin production in the bronchioli and alveoli. 'Pure mucinous' colloid carcinomas, in which the immunohistochemical stains for luminal surface glycoproteins have shown inverted polarity, allow the colloid carcinoma cells to secrete mucin towards the stroma (28). It is therefore possible that such an LKB1 alteration would result in the deregulated polarity of mucin-producing cells, thereby leading to an overproduction of mucin, and an impaired differentiation of goblet cells, thus leading to uncontrolled proliferation. Further investigations are warranted to elucidate these hypotheses.

Homozygous *Lkb1*-deficient mice are lethal at midgestation by defects in the neural tube, mesenchymal cell death and vascular abnormalities (29). Heterozygous mice die of gastric polyps before forming carcinomas (30–32). However, heterozygous mice with other conditional mutants, such as *KRAS* (12), *p53* (33) or *PTEN* (34), show a malignant potential once one of those switches has either been turned on or off. Several oncogene mutations were identified among the mBACs with *LKB1* mutations. LKB1 loss could therefore be oncogenic even under heterozygous inactivation with other oncogenic mutations.

Several drugs have been tested against LKB1-causative tumors. Rapamycin, a macrolide antibiotic that inhibits the mTOR pathway, has been shown to reduce the gastric tumor burden in *Lkb1*(+/−) mice by oral administration (35). Metformin and phenformin, biguanides commonly used to treat diabetes, are some other candidates for treating those tumors. Biguanides inhibit ATP synthesis and thereby cause

a rise in the cellular AMP:ATP ratio, which in turn activates AMPK (36). Several models have also indicated the possible therapeutic use of biguanides for tumors caused by LKB1-AMPK insufficiency (34,37).

In conclusion, frequent *LKB1* mutations were found in mBAC in both PJS cases and sporadic cases. LKB1 inactivation is therefore a possible cause of mBAC tumorigenesis. Furthermore, LKB1 may be a possible target of therapy for mBAC, using LKB1-targeted drugs, such as rapamycin and biguanides delivered either systemically or by airway inhalation.

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Conflict of interest statement

None declared.

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Risk Factors for Tumor Recurrence in Patients With Early-Stage (Stage I and II) Non-small Cell Lung Cancer : Patient Selection Criteria for Adjuvant Chemotherapy According to the Seventh Edition TNM Classification

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

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Original Research

LUNG CANCER

Risk Factors for Tumor Recurrence in Patients With Early-Stage (Stage I and II) Non-small Cell Lung Cancer

Patient Selection Criteria for Adjuvant Chemotherapy According to the Seventh Edition TNM Classification

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

Objectives: **The purpose of this study was to evaluate risk factors for tumor recurrence in patients with completely resected early-stage non-small cell lung cancer (NSCLC).**
Methods: **Between July 1992 and December 2007, 1,967 consecutive patients with stage I and II NSCLC with diagnoses based on the seventh edition TNM classification underwent complete resection. All patients were divided into three groups according to the stage and presence of lymph node metastasis: stage I, patients with stage I, T1-T2aN0M0 disease; stage IIN0, patients with stage II, T2b-T3N0M0, node-negative disease; and stage IIN1, patients with stage II, T1-2N1M0, node-positive disease. Freedom from recurrence rate was estimated using the Kaplan-Meier method, and recurrence risk factors were identified by univariate and multivariate analyses.**
Results: **The 5-year freedom from recurrence rates for stage I, stage IIN0, and stage IIN1 patients were 84%, 61%, and 54%, respectively. By multivariate analyses, three variables (histologic differentiation, vessel invasion, and visceral pleural invasion) in stage I and two variables (adenocarcinoma histology and visceral pleural invasion) in stage IIN0 and stage IIN1 were shown to be independently significant risk factors for recurrence. According to subgroup analyses that combined these risk factors in each group, the 5-year freedom from recurrence rate was 63% for stage I with three risk factors, whereas those for stage IIN0 and stage IIN1 without risk factors were 83% and 78%, respectively.**
Conclusion: **In patients with stage I and II NSCLC, we identified risk factors for recurrence. When these factors are combined, high- and low-risk subgroups can be identified within each group.**
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Abbreviations: BAC = bronchioloalveolar carcinoma; CEA = serum carcinoembryonic antigen; NSCLC = non-small cell lung cancer; VPI = visceral pleural invasion

The most effective treatment of early-stage non-small cell lung cancer (NSCLC) is surgical resection. However, the reported 5-year survival rates of patients with stage I and II disease are approximately 60% to 90% and 30% to 70% after complete resection,[1-17] and not a few patients eventually die because of the disease. Although recent randomized controlled trials have shown a significant survival benefit from cisplatin-based adjuvant chemotherapy for patients with NSCLC stage II or higher,[18,19] whether patients with stage I NSCLC should receive chemotherapy remains controversial.

The seventh edition of the International Union against Cancer TNM for Lung and Pleural Tumors was recently published by the International Association for the Study of Lung Cancer.[20] Better quantification of risk for patients with surgically resected early-stage NSCLC who are categorized based on the seventh edition may improve clinical decision making for adjuvant chemotherapy.

For editorial comment see page 1398

We reviewed a large series of consecutive patients with early-stage NSCLC who underwent complete resection. The purpose of this study was to evaluate patients with stage I and II NSCLC according to the

seventh edition of TNM classification who underwent complete resection and to investigate the risk factors for tumor recurrence, which will help select patients who may benefit from adjuvant therapy or for whom adjuvant therapy is not necessary. To focus on evaluating risk factors for tumor recurrence after resection, we investigated the freedom from recurrence rate instead of overall survival rate.

Materials and Methods

Patients

In total, 2,128 consecutive Japanese patients with pathologic stage I and II NSCLC who were staged based on the seventh edition TNM classification underwent complete resection with lobectomy or a more extensive surgical procedure along with systematic lymph node dissection between August 1992 and December 2007. Among these patients, 161 were excluded either because (1) they had received preoperative or postoperative chemotherapy, radiation therapy, or both (n = 128) or (2) they possessed low-grade pulmonary malignancies (n = 33), including carcinoids, mucoepidermoid carcinomas, or adenoid cystic carcinomas. The remaining 1,967 patients were included in this study. Data collection and analyses were approved, and the need to obtain informed consent from each patient was waived by the institutional review board in April 2010.

Pathologic Evaluations

Histologic type was determined according to the World Health Organization classification,[21] and the histologic grade was diagnosed and categorized as well-differentiated or moderately/poorly differentiated carcinomas according to the degree of structural and cytologic atypia. Disease stages were based on the TNM classification of the International Union against Cancer, seventh edition.[20] Visceral pleural invasion (VPI) and intratumoral vessel invasion was evaluated by hematoxylin-eosin and elastin (Victoria blue-van Gieson) staining.

Patient Follow-up

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

Clinicopathologic Information

Patients with stage I and II NSCLC were divided into three groups according to the stage and presence of lymph node metastasis: stage I, patients with stage I, T1-T2aN0M0 disease; stage IIN0, patients with stage II, T2b-T3N0M0, node-negative disease; and stage IIN1, patients with stage II, T1-2N1M0, node-positive disease. We then reviewed the medical records of each patient for the following clinicopathologic information: age (dichotomized at the median age of 65), sex, smoking history (never or ever smoker), preoperative serum carcinoembryonic antigen (CEA) level (cutoff at the normal upper limit of 5 ng/mL), diameter of the tumor on the resected specimens (≤ 3 cm or > 3 cm in stage I and stage IIN1, and dichotomized at the median size of ≤ 5.5 cm or > 5.5 cm in stage IIN0), histology (adenocarcinoma or non-adenocarcinoma), histologic differentiation (well differentiated or moderately/poorly differentiated), vessel invasion (presence or absence), VPI (as defined in the TNM classification[20];

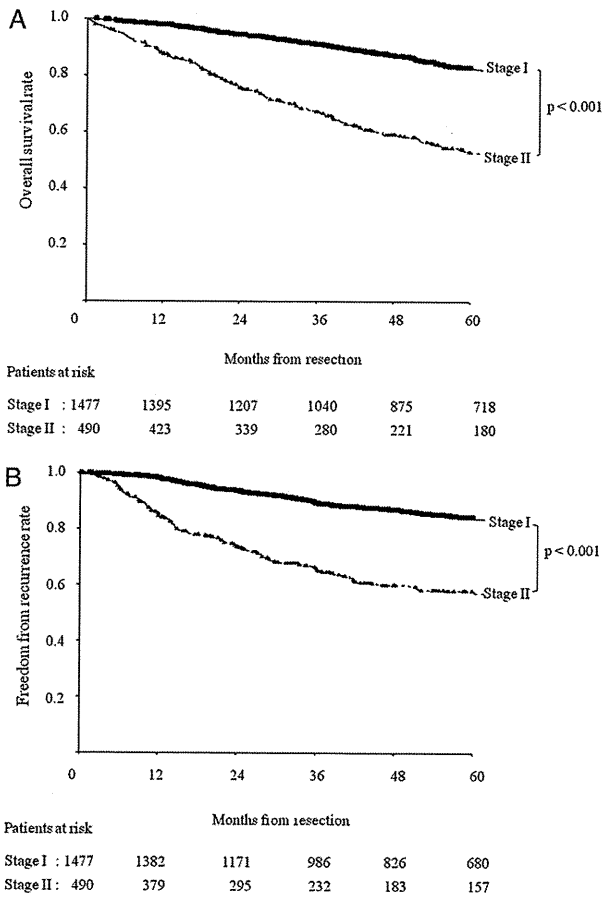

FIGURE 1. A, Overall survival curves for patients with stage I and II non-small cell lung cancer (NSCLC). B, Freedom from recurrence rate curves for patients with stage I and II NSCLC.

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presence or absence), separate tumor nodules in the same lobe as the primary tumor (presence or absence in stage IIN0), number of metastatic N1 nodes (single or multiple in stage IIN1), and the highest level of involved lymph node station (hilar [10] or interlobar [11], or peripheral [12-14] in stage IIN1).

Statistical Analysis

The freedom from recurrence period was calculated in months from the date of resection to the date of the first recurrence or last follow-up. To calculate the freedom from recurrence rate, 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 probabilities were estimated using the Kaplan-Meier method and differences in variables were determined using the log-rank test. Multivariate analyses were performed using Cox proportional hazard regression model. All *P* values reported were two-sided, and the significance level was set at $< .05$.

Results

Of the 1,967 patients included in this analysis, 1,477 were stage I and 490 were stage II, with 247 T2b-T3N0M0 and 243 T1-T2bN1M0. The 5-year overall survival rates and freedom from recurrence rates for patients with stage I and II NSCLC were 82.8% and 52.7% ($P < .001$) (Fig 1A) and 84.2% and 57.6% ($P < .001$) (Fig 1B), respectively.

Freedom From Recurrence Rates and Risk Factors for Tumor Recurrence in Stage I (T1-T2aN0M0)

Table 1 lists the freedom from recurrence rates 5 years after surgical resection according to the clinicopathologic features in patients with stage I. On multivariate analysis using the Cox regression model, histologic differentiation, presence of vessel invasion, and presence of VPI remained statistically significant independent predictors for tumor recurrence in stage I (Table 1).

Freedom From Recurrence Rates and Risk Factors for Tumor Recurrence in Stage IIN0 (T2b-T3N0M0) and Stage IIN1 (T1-T2N1M0)

The 5-year freedom from recurrence rates for stage IIN0 and stage IIN1 were 61.1% and 54.1%, respectively. Tables 2 and 3 list the freedom from

Table 1—*Clinicopathologic Characteristics and Risk Factors for Recurrence in Patients With Stage I NSCLC*

Characteristic	No. of Patients	Freedom From Recurrence Rate, 5 y, %	Univariate *P* Value	Multivariate Analysis		
				HR	95% CI	*P* Value
Overall	1,477	84.2	...			
Age, y			.005[a]			
≤65	724	87.4		1	...	...
>65	753	80.9		1.296	0.988-1.698	.061
Sex			.002[a]			
Female	635	87.5		1	...	...
Male	842	81.6		1.078	0.745-1.559	.69
Smoking habits			<.001[a]			
Never smoker	574	88.9		1	...	...
Ever smoker	903	81.9		1.149	0.764-1.728	.504
CEA			.001[a]			
Within normal range	1,034	86.4		1	...	...
Elevated	441	79.2		1.06	0.804-1.399	1.244
Not examined	2					
Tumor size, cm			<.001[a]			
≤3.0	1,056	87.5		1	...	...
>3.0	421	76		1.244	0.940-1.648	.127
Histologic type			<.001[a]			
Adenocarcinoma	1,112	86.3		1	...	...
Nonadenocarcinoma	365	77.5		1.018	0.750-1.384	.907
Histologic differentiation			<.001[a]			
Well differentiated	538	94.1		1	...	...
Moderately/poorly differentiated	939	77.8		1.916	1.292-2.841	.001[a]
Vessel invasion			<.001[a]			
Absent	902	92.6		1	...	...
Present	575	71.7		2.715	1.952-3.778	<.001[a]
VPI			<.001[a]			
Absent	1,196	88.3		1	...	...
Present	281	66.7		1.768	1.326-2.357	<.001[a]

CEA = preoperative serum carcinoembryonic antigen level, normal upper limit at 5 ng/mL; HR = hazard ratio; NSCLC = non-small cell lung cancer; VPI = visceral pleural invasion.
[a]Significant.