resembled fetal lung.[3] The term "pulmonary blastoma" was first used by Spencer in 1961 based on its embryological similarity to nephroblastoma.[4] Recently, pulmonary blastoma was classified into three types: biphasic pulmonary blastoma, WDFA, and pleuropulmonary blastoma.[5] Biphasic pulmonary blastoma (biphasic PB) is characterized by both malignant epithelial and mesenchymal components, and its structure resembles fetal lung, whereas WDFA is characterized by a monophasic pattern consisting of an epithelial component alone. Pleuropulmonary blastoma, a mesenchymal tumor characterized by embryonic stroma without an epithelial malignant component, is a high-grade aggressive neoplasm of infancy.[6] For that reason pleuropulmonary blastoma is distinguished from pulmonary blastoma of adulthood (biphasic PB and WDFA). In addition to the above histological features, WDFA is characterized by the presence of morules and neuroendocrine cells.[5] Only biphasic pulmonary blastoma is classified as "pulmonary blastoma" in the World Health Organization classification of lung and pleural tumors, and WDFA has been classified as a "variant" of adenocarcinoma.[7]

We have summarized and reviewed the cases reported in Japan, including our own, with emphasis on the clinical features and outcome. Altogether, 25 cases clinically and histopathologically identified as WDFA, including our own, have been reported in Japan from 1985 to 2003.

The clinical data are summarized in Tables 1 and 2 together with the clinical data for WDFA and biphasic PB reported by Koss et al.[5] In the series we reviewed, the mean age at diagnosis was 37 years (range 19–62 years), and there was a unimodal age peak during the third decade. Cases in which the tumor was detected on a chest radiograph as part of a medical checkup accounted for 76%. The tumor size at resection was comparatively small, with a median size of 3.5 cm (range 1.4–12.0 cm). The tumors tended to be localized in the right lung (especially in the upper lobe). The sex ratio was a 2:3 (males 10 cases, females 15 cases). Moreover, among the 24 cases in which the pathological stage was reported, there were two cases with N1 lymph node metastasis at the time of resection, and one of them had a distant metastasis to an eye at the time of diagnosis. There were no N2-positive cases, and almost all of the cases were N0 (22 cases, 88%). The follow-up period was reported for 22 cases. The median follow-up period was 36 months (5–120 months), and two (8%) of the patients died of their tumor.

In a comparison of the outcome of WDFA and biphasic PB, Koss et al. showed that the median survival of patients with WDFA was significantly longer than that of patients with biphasic PB,[5] and Larsen and Sorenson et al. reported a significantly longer median survival for patients with WDFA (34 months) than with

Table 1 Summary of 25 cases reported in Japan

No.	Year	Author	Age (years)	Sex	Size (cm)	pT	pN	Stage	Recurrence	Outcome
1	1985	Mitsuoka	39	M	7.0	2	0	I		Alive at 72 months
2	1986	Ogawa	33	F	6.0	2	0	I	+	Alive at 55 months
3	1987	Tanimura	27	F	9.0	2	0	I		Alive at 49 months
4	1992	Sato	32	F	8.0	2	0	I		Alive at 5 months
5	1992	Higashiyama	52	M	6.0	2	0	I	+	Dead at 28 months
6	1992	Higashiyama	62	M	2.0	1	0	I	ND	Alive at 42 months
7	1992	Higashiyama	36	M	2.4	1	0	I	ND	Alive at 88 months
8	1993	Shimada	31	F	4.5	2	0	I	ND	ND
9	1995	Fujino	33	F	9.0	2	1	II		Alive at 12 months
10	1996	Okano	33	F	5.0	2	0	I		Alive at 11 months
11	1997	Izumi	32	F	3.0	1	0	I	+	Alive at 33 months
12	1997	Matsumoto	30	F	3.2	2	0	I		Alive at 48 months
13	1998	Nakatani	35	M	1.4	1	0	I		Alive at 24 months
14	1998	Nakatani	35	F	2.2	1	0	I		Alive at 24 months
20	1998	Nakatani	39	M	2.5	1	0	I		Alive at 120 months
16	1998	Nakatani	40	F	3.0	1	0	I		Alive at 48 months
19	1998	Nakatani	35	F	3.0	1	0	I		Alive at 108 months
18	1998	Nakatani	33	F	3.5	2	0	I		Alive at 72 months
17	1998	Nakatani	45	M	4.6	2	0	I		Alive at 48 months
15	1998	Nakatani	55	F	5.0	2	1	IV		Dead at 24 months
21	1998	Nakatani	19	M	1.5	ND	ND	ND	ND	ND
22	2001	Tatebayashi	27	F	2.6	1	0	I		Alive at 8 months
23	2001	Sawamoto	24	F	12.0	2	0	I	ND	ND
24	2003	Kawai	58	M	3.2	2	0	I		Alive at 36 months
25		Present case	36	M	4.1	2	0	I		Alive at 38 months

ND, no description

Table 2 Comparative clinical features of WDFA and biphasic pulmonary blastoma

Parameter	Japan: WDFA	Koss et al.	
		WDFA	Biphasic PB
No. of cases	25	28	24
Mean age (years)	37	33	39
Male/female	10/15	13/15	11/13
Percent asymptomatic	76	57	17
Tumor size (cm): mean/median/range	4.5/3.5/1–12	4.5/3.8/1–10	10.1/8.2/2–27
Location (right/left)	15/10 (3/2)	ND	ND
Pathological stage I	22	20	19
Lymph node metastasis	2 (8%)	3 (14%)	12 (52%)
N0/N1/N2	(22/2/0)	ND	ND
Median follow-up (months)	39	95	13
No. who died of their tumor	2 (8%)	3 (14%)	12 (52%)

WDFA, well-differentiated fetal adencarcinoma; PB, pulmonary flastoma; ND, no description

biphasic PB (11 months).[8] Koss et al. reported 6 cases (29%) of recurrent tumor during follow-up of 21 cases of WDFA. Three patients were treated surgically and were alive without tumor after follow-up periods of 33, 101, and 153 months; three patients (14%) died of their tumor. Of 23 patients followed up for biphasic blastomas, 10 (43%) were found to have tumor recurrence at 1–11 months (mean 4.6 months) after the initial diagnosis, and 9 of the 10 patients with recurrent disease died. Altogether, 12 (52%) of the 23 patients died of their tumor; and 9 of the 12 deaths occurred within 1 year after thoracotomy. As described above, WDFA has the characteristic that mediastinal lymph node metastasis is rare, and the prognosis is better than for biphasic pulmonary blastoma. However, it is difficult to diagnose a lung tumor as WDFA during the preoperative examination, so standard surgical procedures would be necessary as usual.

A recent study identified molecular abnormalities in the pathogenesis of WDFA and detected *p53* gene mutation and overexpression of p53 protein. Nakatani et al. and Sekine et al. reported aberrant nuclear/cytoplasm expression of β-catenin and hypothsized that β-catenin mutation is involved in epithelial cell overgrowth and morule formation in pulmonary blastomas, and that WDFA forms as a result of one-sided epithelial outgrowth from biphasic blastoma due to β-catenin mutation at an early stage of tumorigenesis.[9,10] The molecular-biological features of pulmonary blastomas, including the differences of WDFA and biphasic PB, will continue to be become clear.

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Int. J. Cancer: **118,** 2498–2504 (2006)

Frequent *EGFR* mutations in noninvasive bronchioloalveolar carcinoma

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Mutations of the epidermal growth factor receptor gene (*EGFR*) have been reported to be present in a considerable fraction of lung adenocarcinomas showing dramatic response to EGFR tyrosine kinase inhibitors. To clarify pathogenic significance of the mutations for the development of lung adenocarcinoma, we investigated stage I lung adenocarcinomas for the mutations. First, 107 cases of macrodissected stage I adenocarcinomas were examined for mutations in exons 18–21 of the *EGFR* gene. *EGFR* mutations were detected in 36 of the 107 cases (34%). In particular, among the stage I cases, the mutations were detected in 17 of 42 small-sized adenocarcinomas (≤2 cm in diameter) (40%), including 7 of 11 noninvasive bronchioloalveolar carcinomas (BACs) (64%) and 7 of 25 invasive adenocarcinomas with BAC components (28%). Second, 26 cases of laser capture microdissected small-sized adenocarcinomas, including 9 cases in the first analysis, were examined for the mutations. Reanalysis of microdissected materials in the 9 cases identified the mutations in 2 more adenocarcinomas with BAC components. Moreover, in the analysis of the other 17 microdissected materials, *EGFR* mutations were detected in 7 of 12 BACs (58%) and in 3 of 5 adenocarcinomas with BAC components (60%). *EGFR* mutations are present frequently in BACs, and are thus likely to be a critical genetic alteration for the formation of noninvasive lung adenocarcinoma.

Key words: *EGFR* mutation; small-sized lung adenocarcinoma; bronchioloalveolar carcinoma (BAC)

Epidermal growth factor receptor (EGFR) is a member of a family comprised of 4 homologous receptors, including EGFR (ErbB1), HER-2/neu (ErbB2), HER-3 (ErbB3) and HER-4 (ErbB4). Ligand binding to EGFR leads to receptor tyrosine kinase (TK) activation and a series of downstream signaling activation that mediates increase in cellular proliferation, migration, invasion and suppression of apoptosis.[1] In 2004, 2 groups simultaneously reported that most nonsmall cell lung cancer (NSCLC) patients who were responsive to gefitinib, an EGFR TK inhibitor, had somatic mutations in the kinase domain of the *EGFR* gene in their tumor cells.[2,3] Subsequently, it was revealed that *EGFR* mutations are present in a considerable fraction of NSCLC and occur more frequently in East Asian patients, including Japanese, than in Caucasian patients.[4–10] Furthermore, the incidence of *EGFR* mutations was significantly high in female patients with adenocarcinoma without smoking histories. This population corresponded to responders to gefitinib. These data indicate that *EGFR* mutations and/or responsiveness to gefitinib are likely to be associated with a specific subset of lung adenocarcinoma. However, there is still little evidence about when the mutations occur during the development of lung adenocarcinoma and whether the mutations are responsible for a specific phenotype in lung adenocarcinoma.

In 1995, Noguchi *et al.*[11] classified small-sized lung adenocarcinomas of ≤2 cm in diameter into 6 histological subtypes, as follows: type A, localized bronchioloalveolar carcinoma (LBAC); type B, LBAC with foci of alveolar structural collapse; type C, LBAC with foci of active fibroblastic proliferation; type D, poorly differentiated adenocarcinoma; type E, tubular adenocarcinoma; and type F, papillary adenocarcinoma with compressive and destructive growth. Types A, B and C grow by replacing the pulmonary alveolar structure (replacing growth type), whereas types D, E and F grow destructively, without such a replacement. In 1999, "bronchioloalveolar carcinoma (BAC)" was newly defined as a subtype of lung adenocarcinoma in the World Health Organization (WHO) classification of lung cancers, in which BAC was defined as being a true noninvasive cancer without evidence of stromal, vascular or pleural invasion.[12] This definition was preserved in the 2004 WHO classification.[13] Among the replacing growth types of adenocarcinomas, types A and B are true noninvasive BAC and type C is an invasive adenocarcinoma with BAC component; thus, types A and B correspond to BAC in the WHO classification. Accordingly, type A tumors are assumed to sequentially progress through type B to type C. Aoyagi *et al.*[14] reported that accumulation of loss of heterozygosity (LOH) in crucial chromosome regions occurred stepwise during this sequential progression. The results indicate that a fraction of genetic alterations are involved in the progression from noninvasive to invasive lung adenocarcinoma. Since *EGFR* mutations are present in a considerable fraction of lung adenocarcinomas, it is important to clarify whether *EGFR* mutations are present in noninvasive stages or in invasive stages of lung adenocarcinoma and to which histological subtypes of lung adenocarcinoma the mutations are associated with.

In the present study, we first investigated 107 cases of stage I lung adenocarcinoma for *EGFR* mutations. The mutations were detected in 34% of the stage I cases. Then, to address the above unresolved issues, we further analyzed small-sized adenocarcinomas (≤2 cm in diameter) of stage I cases and compared the mutations with histological subtypes of adenocarcinoma using histological classification reported by Noguchi *et al.* *EGFR* mutations were detected in a majority of type A and B adenocarcinomas. Thus, it was indicated that *EGFR* mutations may contribute to the development of BAC and are one of the early genetic alterations in multistage carcinogenic processes of lung adenocarcinoma.

Material and methods

Patients and tissues

Primary tumors were obtained from 124 patients with stage I lung adenocarcinoma, including 59 patients with small-sized lung adeno-

Abbreviations: BAC, bronchioloalveolar carcinoma; EGFR, epidermal growth factor receptor; LBAC, localized bronchioloalveolar carcinoma; LOH, loss of heterozygosity; NSCLC, nonsmall cell lung cancer; PCR, polymerase chain reaction; SDS, sodium dodecyl sulfate; TK, tyrosine kinase.

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Received 5 August 2005; Accepted 4 October 2005
DOI 10.1002/ijc.21670
Published online 13 December 2005 in Wiley InterScience (www.interscience.wiley.com).

TABLE I – CORRELATION BETWEEN *EGFR* MUTATIONS AND CLINICOPATHOLOGICAL CHARACTERISTICS IN PATIENTS WITH STAGE I LUNG ADENOCARCINOMA

Clinicopathological feature	Subset	Number of patients	*EGFR* mutation (%)		*p*-Value[1]
			+	–	
Gender	Male	61	16 (26)	45 (74)	0.07
	Female	46	20 (43)	26 (57)	
Age	≤63	54	22 (41)	32 (59)	0.15
	>63	53	14 (26)	39 (74)	
Smoking history	Smoker	62	11 (18)	51 (82)	<0.0001
	Nonsmoker	45	25 (56)	20 (44)	
T stage	T1	70	25 (36)	45 (64)	0.67
	T2	37	11 (30)	26 (70)	
Tumor size	≤2 cm	42	17 (40)	25 (60)	0.30
	>2 cm	65	19 (29)	46 (71)	
Differentiation	Poorly	15	4 (25)	12 (75)	0.55 (poorly *vs* well),
	Moderately	37	12 (33)	24 (67)	0.75 (poorly *vs* mod.)
	Well	55	20 (36)	35 (64)	
KRAS mutation	+	12	0 (0)	12 (100)	0.008
	–	95	36 (38)	59 (62)	
p53 mutation	+	35	9 (26)	26 (74)	0.28
	–	72	27 (38)	45 (63)	

[1]Fisher's exact test.

carcinoma of ≤2 cm in their maximum diameter. All of the patients underwent curative pulmonary resections at the National Cancer Center Hospital, Tokyo, Japan, from December 1986 to December 2000. None of the patients received neoadjuvant or adjuvant chemotherapy, including treatment with gefitinib and radiotherapy before or after surgery. The tumors were pathologically diagnosed according to the tumor-node-metastasis classification of malignant tumors.[15] In addition, all of the small-sized adenocarcinomas were histologically classified into 6 groups according to the histological classification of small-sized adenocarcinoma of the lung, which was previously reported by Noguchi *et al.*[11]

For the first series of analysis, 107 cases, including 42 with small-sized adenocarcinomas, were used. Clinicopathological characteristics of these patients are summarized in Table I. The median follow-up period of these patients was 63 months (range, 4–110 months). Tumors analyzed in the first series were macrodissected and stored at −80°C until DNA extraction. Their genomic DNAs were prepared by the method described previously[16] or by a QIAamp DNA mini kit (Qiagen, Tokyo, Japan). Cancer cells of 26 cases with small-sized lung adenocarcinoma, which consisted of 9 cases analyzed in the first series and an additional 17 cases, were collected by laser capture microdissection. The tumor specimens obtained from these cases were fixed with methanol and embedded in paraffin. Three or four 8-μm-thick sections from each specimen were deparaffinized and stained with hematoxylin. The stained sections were dried and cancer cells were microdissected using the Pixcell Laser Capture microdissection system (Arcturus Engineering, Mountain View, CA). A total of 1,000–5,000 cancer cells were microdissected from each specimen, and their genomic DNAs were isolated by sodium dodecyl sulfate (SDS)/proteinase K digestion and phenol/chloroform extraction as described previously.[17]

Mutational analysis of the EGFR *gene*

Exons 18–21 of the *EGFR* gene were examined for mutations by genomic polymerase chain reaction (PCR) amplification and direct sequencing of PCR products. Ten nanograms of DNA extracted from the macrodissected materials was used for PCR amplification. For the microdissected materials, nested PCR was carried out after initial PCR using 100 pg of DNA. The primer sets for the initial PCR were as follows: exon 18, 5′-CAAATGAGCTGGCAAGTGCCGTGTC-3′ (forward) and 5′-GAGTTTCCCAAACACTCAGTGAAAC-3′ (reverse); exon 19, 5′-GCAATATCAGCCTTAGGTGCGGCTC-3′ (forward) and 5′-CATAGAAAGTGAACATTTAGGATGTG-3′ (reverse); exon 20, 5′-CCATGAGTACGTATTTTGAAACTC-3′ (forward) and 5′-CATATCCCCATGGCAAACTCTTGC-3′ (reverse); exon 21, 5′-CTAACGTTCGCCAGCCATAAGTCC-3′ (forward) and 5′-GCTGCGAGCTCACCCAGAATGTCTGG-3′ (reverse). The internal primer sets for nested PCR were as follows: exon 18, 5′-CAAGTGCCGTGTCCTGGCACCCAAGC-3′ (forward) and 5′-CCAAACACTCAGTGAAACAAAGAG-3′ (reverse); exon 19, 5′-CCTTAGGTGCGGCTCCACAGC-3′ (forward) and 5′-CATTTAGGATGTGGAGATGAGC-3′ (reverse); exon 20, 5′-GAAACTCAAGATCGCATTCATGC-3′ (forward) and 5′-GCAAACTCTTGCTATCCCAGGAG-3′ (reverse); exon 21, 5′-CAGCCATAAGTCCTCGACGTGG-3′ (forward) and 5′-CATCCTCCC-CTGCATGTGTTAAAC-3′ (reverse). Thirty-five cycles (for initial PCR) or 30 cycles (for nested PCR) of 95°C (30 sec) for denaturation, 58°C (1 min) for annealing and 72°C (30 sec) for extension were performed to amplify DNA fragments. PCR products were cycle-sequenced using an ABI PRISM Dye Terminator Cycle Sequencing Ready Reaction kit (Applied Biosystems, Foster City, CA) and an ABI PRISM 3100 DNA Sequencer (Applied Biosystems).

Mutational analysis of the p53 *gene and the* KRAS *gene*

We previously examined the 107 macrodissected materials for mutations of the *p53* gene and the *KRAS* gene.[18] Briefly, the *p53* gene (exons 2–11) was amplified by genomic PCR. PCR products with variant peaks detected by the WAVE DNA Fragment Analysis System and WAVEMAKER Software 4.0 (Transgenomic, Omaha, NE) were purified by a QIAquick PCR Purification kit (Qiagen) for sequencing. The *KRAS* gene (exons 1 and 2) was also amplified by genomic PCR and directly sequenced. Cycle sequencing was performed using the ABI PRISM Dye Terminator Cycle Sequencing Ready Reaction kit (Perkin-Elmer, Norwalk, CT) and the ABI PRISM 310 DNA Sequencer (Perkin-Elmer).

Microdissected materials were also analyzed for *KRAS* mutations. One-hundred picograms of DNA was used for initial PCR amplification. Nested PCR was carried out after initial PCR. The primer sets for initial PCR were as follows: exon 1, 5′-GGTACTGGTGGAGTATTTGAT-3′ (forward) and 5′-ATGGTCAGAGAAACCTTTATCT-3′ (reverse); exon 2, 5′-CCTTTTTTGAAGTAAAAGGTGC-3′ (forward) and 5′-ATCCCCCAAGAACTTCATTTAT-3′ (reverse). The internal primer sets for nested PCR were as follows: exon 1, 5′-TGGTGGAGTATTTGATAGTGTA-3′ (forward) and 5′-ATCTGTATCAAAGAATGGTCCT-3′ (reverse); exon 2, 5′-GGTGCACTGTAATAATCCAGA-3′ (forward) and 5′-ATTACTCCTTAATGTCAGCTTAT-3′ (reverse). Thirty-five cycles (for initial PCR) or 30 cycles (for nested PCR) of 95°C (1 min) for denaturation, 60°C (1 min) for annealing and 72°C (1 min) for extension were performed to amplify DNA fragments. PCR products were cycle-sequenced

using an ABI PRISM Dye Terminator Cycle Sequencing Ready Reaction kit (Applied Biosystems) and an ABI PRISM 3100 DNA Sequencer (Applied Biosystems).

Statistical analysis

Fisher's exact test was used to assess the association of *EGFR* mutations with clinicopathological factors. The association was further examined by multivariate odds ratios adjusted by each other (logistic regression analysis). Overall survivals of the patients with and without *EGFR* mutations were compared by the Kaplan–Meier curves and the log-rank test. In addition, the effect of the mutations on survival was also investigated using the Cox proportional hazards regression model adjusted by other potential prognostic factors shown in Table I. A p value of <0.05 was considered to be statistically significant. Statistical analysis was performed using the SAS (version 8.02; SAS Institute, Cary, NC).

Results

Correlation between EGFR *mutations and clinicopathological characteristics in stage I lung adenocarcinoma*

One hundred and seven macrodissected stage I lung adenocarcinomas were examined for mutations in exons 18–21 of the *EGFR* gene by genomic PCR and direct sequencing. *EGFR* mutations were detected in 36 cases (34%) (Table I). *EGFR* mutations were significantly more frequent in nonsmokers (56%) than in smokers (18%) ($p < 0.0001$). Female patients (43%) tended to have *EGFR* mutations more frequently in their tumors than male patients (26%), although the difference in frequency did not reach the statistical significance ($p = 0.07$). There was no significant correlation of *EGFR* mutations with age, T stage, tumor size ($\leq$2 cm *vs.* >2 cm) and differentiation.

All tumors in this series of stage I lung adenocarcinomas had been previously examined for mutations in exons 1 and 2 of the *KRAS* gene and in exons 2–11 of the *p53* gene.[18] *KRAS* mutations were detected in 12 of 107 cases (11%): 10 in codon 12, 1 in codon 13 and 1 in codon 61. *p53* mutations were detected in 37 of the 107 cases. Because 2 of the 37 cases showed a silent *p53* mutation, we counted 35 of the 107 (33%) as cases with *p53* mutation in this analysis. No *EGFR* mutation was detected in tumors with *KRAS* mutations, indicating a mutually exclusive correlation ($p = 0.008$). In contrast, there was no significant correlation or inverse correlation between *EGFR* mutation and *p53* mutation ($p = 0.28$).

The association of *EGFR* mutations with the clinicopathological characteristics was also evaluated by logistic regression analysis to account for the effect of the different variables. Nonsmoking was independently associated with *EGFR* mutations ($p = 0.003$; odds ratio, 6.83; 95% confidence interval (C. I.), 1.92–24.32), whereas the other factors shown in Table I were not.

We then analyzed the effect of *EGFR* mutations on survival of the 107 patients with stage I lung adenocarcinoma. Kaplan–Meier survival estimates showed that *EGFR* mutations did not affect prognosis of the patients ($p = 0.60$; log-rank test) (Fig. 1). A multivariate Cox proportional hazard regression analysis of all of the clinicopathological and molecular factors also showed that *EGFR* mutations were not an unfavorable prognosis factor independent of other factors ($p = 0.85$; hazard ratio, 1.11; 95% C. I., 0.38–3.24).

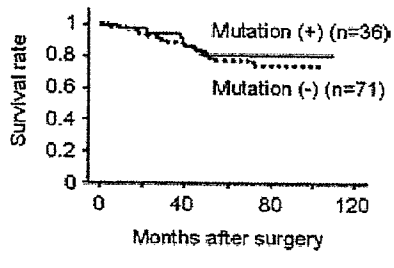

FIGURE 1 – Survival curves for patients with stage I lung adenocarcinoma classified according to *EGFR* mutations. The resulting curves were compared using log-rank test ($p = 0.60$).

EGFR *mutations in small-sized adenocarcinoma of the lung*

It was noted that the frequency of *EGFR* mutations in small-sized adenocarcinomas (17/42, 40%) was a little higher than that in stage I cases in total. This result may indicate that *EGFR* mutations occur early in the development of stage I lung adenocarcinoma. Therefore, according to the histological classification of small-sized adenocarcinoma reported by Noguchi *et al.*,[11] we further analyzed the frequency of *EGFR* mutations as well as those of *KRAS* and *p53* mutations (Table II). Among 11 BACs (type A and B tumors), *EGFR* mutations were detected in 1 of 3 type A (33%) and in 6 of 8 type B tumors (75%), whereas *KRAS* mutation was detected in only 1 type A tumor and not detected in any type B tumors. Of 25 adenocarcinomas with BAC components (type C tumors), 7 (28%) had *EGFR* mutations and 4 (16%) had KRAS mutations. All *p53* mutations were detected in type C, D and F tumors, whereas none of the type A and B tumors had *p53* mutations.

EGFR *mutations in noninvasive and invasive adenocarcinomas*

It is generally accepted that noninvasive adenocarcinomas further progress to invasive ones by accumulation of additional genetic alterations. However, unexpectedly, the *EGFR* gene was less frequently mutated in invasive adenocarcinomas with BAC components (type C tumors) than in BACs (type A and B tumors). Since type C tumors contain a large population of fibroblasts and other noncancerous cells, it was possible that *EGFR* mutations were masked by contaminating noncancerous cells in tumor samples. Therefore, we further analyzed 26 cases of type A, B and C tumors, in which cancer cells were obtained by laser capture microdissection.

Among the 42 cases of small-sized adenocarcinomas in the first series, 9 cases were reevaluated using microdissected materials (Table III). Seven of the 9 cases showed the same results as macrodissected materials in the first analysis, that is, 5 cases had *EGFR* mutations and 2 cases did not in both materials. However, in the remaining 2 cases, both of which were classified into type C, *EGFR* mutations were detected only in microdissected materials and not in macrodissected ones. These 2 tumors were rather small, 1.0 and 1.4 cm in size, respectively, and both harbored the leucine to arginine mutation at codon 858 (L858R) in exon 21. Photographs of microdissection and sequence chromatograms obtained from one of these tumors are shown in Figures 2*a* and 2*c*, respectively. Among the 5 cases in which *EGFR* mutations were detected in both macrodissected and microdissected materials, there were 3 cases whose sequence chromatograms showed the increase in the ratio of mutant allele to wild-type allele in microdissected materials compared with macrodissected materials. All of the 3 tumors were <1.5 cm in size, and 1 was classified into type B and the other 2 were into type C. Representative photographs of microdissection and sequence chromatograms are shown in Figures 2*b* and 2*d*, respectively. Thus, *EGFR* mutations were masked in some cases by the contamination of noncancerous cells in the macrodissected materials, in particular, in the small-sized type C tumors.

To confirm the results from the macrodissected small-sized adenocarcinomas in the first series, we examined an additional 17 small-sized adenocarcinomas for *EGFR* mutations using laser capture microdissection. The 17 cases consisted of 5 cases of type A, 7 of type B and 5 of type C. Ten of the 17 cases (59%) had *EGFR* mutations. As in the analysis of macrodissected materials, *EGFR* mutations were frequently detected in type A and B tumors (7 of 12, 58%). Moreover, *EGFR* mutations were also frequently detected in type C tumors (3 of 5, 60%). These results suggest that

TABLE II - FREQUENCY OF *EGFR*, *KRAS*, *P53* MUTATIONS IN SMALL-SIZED ADENOCARCINOMAS OF THE LUNG

Variable	Frequency (%)						
	Total	Subtype					
		A	B	C	D	E	F
EGFR mutation	17/42 (40)	1/3 (33)	6/8 (75)	7/25 (28)	1/4 (25)	0/0	2/2 (100)
KRAS mutation	6/42 (14)	1/3 (33)	0/8 (0)	4/25 (16)	1/4 (25)	0/0	0/2 (0)
p53 mutation	12/42 (29)	0/3 (0)	0/8 (0)	9/25 (36)	2/4 (50)	0/0	1/2 (50)

TABLE III - FREQUENCY OF *EGFR* AND *KRAS* MUTATIONS IN MACRODISSECTED AND MICRODISSECTED SMALL-SIZED ADENOCARCINOMAS OF THE LUNG

Material	Frequency (%)							
	EGFR mutation				*KRAS* mutation			
	Total	Subtype			Total	Subtype		
		A	B	C		A	B	C
Macrodissected (n = 27)	9/27 (33)	0/1 (0)	4/6 (67)	5/20 (25)	4/27 (15)	0/1 (0)	0/6 (0)	4/20 (20)
Macro- and Microdissected (n = 9)								
Macrodissected	5/9 (56)	1/2 (50)	2/2 (100)	2/5 (40)	1/9 (11)	1/2 (50)	0/2 (0)	0/5 (0)
Microdissected	7/9 (78)	1/2 (50)	2/2 (100)	4/5 (80)	1/9 (11)	1/2 (50)	0/2 (0)	0/5 (0)
Microdissected (n = 17)	10/17 (59)	2/5 (40)	5/7 (71)	3/5 (60)	2/17 (12)	1/5 (20)	0/7 (0)	1/5 (20)

EGFR mutations are present in the majority of small-sized adenocarcinoma, irrespective of noninvasive or invasive ones.

We also examined these 17 microdissected materials for *KRAS* mutations and found the mutations in 2 tumors without *EGFR* mutations. One of the tumors with *KRAS* mutation belonged to type A and the other belonged to type C. No *KRAS* mutation was detected in 7 type B tumors, consistent with the results of the first series.

In 4 of the microdissected type C tumors, we were able to separately collect cancer cells from the replacing growth (noninvasive) component and those from the central fibrotic area (invasive component) of a single tumor, and examined for *EGFR* and *KRAS* mutations in each of the components independently. Three of the 4 tumors had *EGFR* mutations in the cancer cells from both components, while the remaining 1 tumor had a *KRAS* mutation also in the cancer cells from both components. There was no tumor that showed mutations only in the invasive cancer cells or in the noninvasive ones (data not shown).

Types of EGFR *mutations in stage I lung adenocarcinoma*

In a total of 124 stage I lung adenocarcinomas analyzed in the present study, 52 *EGFR* mutations were detected in 48 cases. All types of mutations identified in the present study are shown in Table IV. There were 28 (54%) in-frame deletions, 4 (8%) in-frame duplications/insertions and 20 (38%) point mutations, but no frameshift mutations and nonsense mutations. Twenty-four of the 28 deletions were simple deletions of 5 amino acid residues from codon 746 to 750, and 1 deletion was a simple deletion of 5 amino acid residues from codon 747 to 751. The remaining 3 deletions were coupled with 1 or 2 amino acid substitutions. Among the 20 point mutations, the leucine to arginine mutation at codon 858 (L858R) in exon 21 was found in 11 tumors. Two tumors with the L858R mutation had other point mutations at codon 709 in exon 18, which were the glutamic acid to lysine mutation (E709K) and the glutamic acid to glycine mutation (E709G), respectively. Two tumors with deletions also had other point mutations in exon 20, which were the serine to isoleucine mutation at codon 768 (S768I) and the threonine to methionine mutation at codon 790 (T790M), respectively.

Discussion

One hundred and seven macrodissected stage I lung adenocarcinomas were initially examined for *EGFR* mutations, resulting in the detection of the mutations in 36 tumors (34%). In previous studies investigating NSCLC for *EGFR* mutations, no correlation was observed between *EGFR* mutations and stage of the disease.[4–7] The frequency of *EGFR* mutations in our first analysis of stage I adenocarcinomas was also consistent with those in several studies for *EGFR* mutations in various stages of adenocarcinomas in East Asia, including Japan.[4,5,8,9] These results indicate that *EGFR* mutations occur early in the development of lung adenocarcinoma. Indeed, we demonstrated here that, even in small-sized adenocarcinomas among stage I cases, 40% (17 of 42) had *EGFR* mutations. Therefore, we further analyzed the association of the mutations with histological features of small-sized adenocarcinoma. Among replacing growth types of small-sized adenocarcinoma, *EGFR* mutations were detected in 1 of 3 (33%) type A and 6 of 8 (75%) type B in the first analysis of macrodissected materials. The incidences were reproduced in the second analysis of microdissected materials [2 of 5 (40%) type A and 5 of 7 (71%) type B tumors, respectively]. On the other hand, in type C tumors, *EGFR* mutations were detected in 7 of 25 (28%) tumors in the first analysis. When 5 cases of type C in the first analysis were reanalyzed using microdissected materials, *EGFR* mutations were detected in 2 more cases. Moreover, the mutations were detected in 3 (60%) of the additional 5 type C microdissected materials. Thus, the lower frequency of *EGFR* mutations in type C tumors than in type A and B tumors in the first analysis is likely to be due to masking by the contamination of noncancerous cells in the macrodissected type C tumors. These results suggest that *EGFR* mutations are present in the majority of replacing growth type of small-sized adenocarcinomas. In addition, given a sequential progression from noninvasive (types A and B) to invasive tumors (type C) in the concept of multistage carcinogenesis of lung adenocarcinoma,[19] *EGFR* mutations are suggested to be involved in the development of noninvasive adenocarcinoma before progression to invasive adenocarcinoma. Indeed, when we separately examined cancer cells from the replacing growth (noninvasive) component and from the central fibrotic area (invasive component), *EGFR* mutations were always detected in both components and there was no tumor with the mutation only in the invasive component. These findings further support our suggestion. However, because more than 70% of the type B tumors had *EGFR* mutations, the mutations might be more strongly associated with the formation of type B tumors, featuring LBAC with foci due to collapse of the alveolar structure. If so, the results may imply that type C tumors are not always progressed sequentially from type A tumors through type B tumors. In other words, it can be said that BACs are preinvasive lesions for a subset of invasive adenocarcinomas.

There were some previous studies evaluating the correlation between *EGFR* mutations and histological features of lung adenocarcinoma. Yatabe *et al.*[20] reported that *EGFR* mutations were

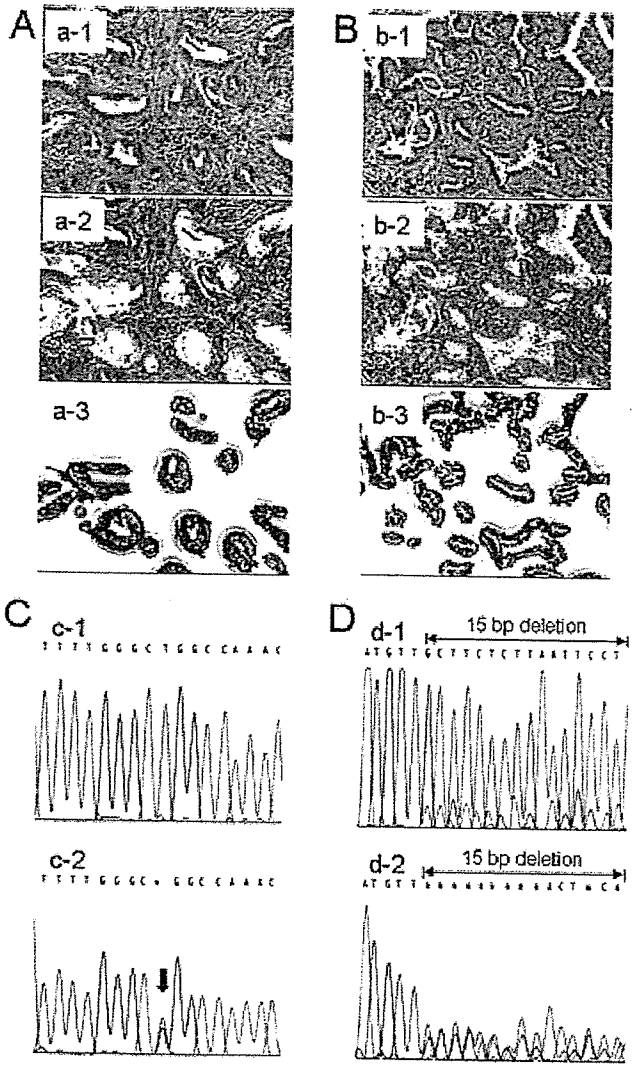

FIGURE 2 – Representative photographs of laser capture microdissection and sequence data showing difference between macrodissected materials and microdissected materials obtained from the same patients. (*a*) Microdissection of cancer cells from a 14 mm-sized type C adenocarcinoma (magnification ×100). (*b*) Microdissection of cancer cells from a 10-mm-sized type B adenocarcinoma (magnification ×100). (*a-1*) and (*b-1*) show central regions of the tumors in hematoxylin-stained tissue sections. (*a-2*) and (*b-2*) show the same sections after microdissection. (*a-3*) and (*b-3*) show the cells captured on the transfer films. (*c*) Sequence data obtained from the type C tumor shown in (*a*). The sequence chromatogram from macrodissected cancer cells is shown in (*c-1*), and that from microdissected cancer cells is shown in (*c-2*). A heterozygous point mutation at nucleotide 2573 (T to G) was detected in microdissected materials (down-arrow) but not in macrodissected ones. (*d*) Sequence data obtained from the type B tumor shown in (*b*). The sequence chromatogram from macrodissected cancer cells is shown in (*d-1*), and that from microdissected cancer cells is shown in (*d-2*). Although a heterozygous in-frame deletion was detected in both materials, peaks of a mutant allele in macrodissected materials are smaller than those in microdissected materials.

specifically involved in terminal respiratory unit-type adenocarcinoma, which corresponds to the majority of nonmucinous BACs. Furthermore, Marchetti *et al.*[6] reported that the histologic type of BAC was independently associated with *EGFR* mutations, in their multivariable analysis. Thus, most of the previous reports demonstrated the preferential occurrence of *EGFR* mutations in tumors with features of BAC.[2,6,21,22] However, in those studies, adenocarcinomas with BAC features contained various extents of invasive regions and often belonged to advanced stages. Furthermore, BAC and adenocarcinoma with BAC features might be confused. There were a few reports demonstrating the incidence of *EGFR* mutations in BAC according to the WHO classification. Kosaka *et al.*[4] reported that 3 of 5 BACs defined by the WHO criteria had *EGFR* mutations. On the other hand, Shigematsu *et al.*[7] reported that none of the 7 BACs collected in the United States had *EGFR* mutation. Thus, it has been controversial whether *EGFR* mutations are commonly present in strictly defined BAC, although the different incidence between their reports might be, in part, attributable to racial difference. In the present study, type A and B adenocarcinomas, which have absolutely no evidence of invasion, correspond to BAC in the WHO classification. Thus, this is the first report, to our knowledge, to analyze a comparable number of BAC for *EGFR* mutations and to demonstrate frequent occurrence of them.

The present study also showed a mutually exclusive correlation between *EGFR* mutations and *KRAS* mutations, consistent with the results reported previously.[4,6,7] *KRAS* mutations are known to be associated with exposure to carcinogens in tobacco smoke and to play an important role in the pathogenesis of lung adenocarcinoma.[23,24] In our analysis, 13 of 14 tumors with *KRAS* mutation were obtained from smokers, whereas *EGFR* mutations were significantly more frequent in nonsmokers. However, there was no significant difference in tumor size, differentiation and patient prognosis between the tumors with *EGFR* mutations and those with *KRAS* mutations (data not shown). In addition, it is of note that in a total of the 59 small-sized adenocarcinomas analyzed, 37 (63%) had either *EGFR* or *KRAS* mutations. When limited to the 17 cases of microdissected materials analyzed, 12 (71%) had either mutation. These results indicate that either *EGFR* or *KRAS* mutation may be required for the development of the majority of small-sized adenocarcinomas. Furthermore, separate analysis of cancer cells from noninvasive component and from invasive component in microdissected type C tumors showed that *KRAS* mutation, as well as *EGFR* mutation, was present in both components. Therefore, *KRAS* mutations may also contribute to the formation of noninvasive adenocarcinoma. However, it remains unclear whether biological behavior is necessarily equal in the tumors with *EGFR* mutation and those with *KRAS* mutation. In this study, we also obtained another interesting result that *KRAS* mutations were not detected in any type B tumors analyzed. This result was extremely different from the high incidence of *EGFR* mutations in type B tumors. Although *EGFR* mutations are likely to contribute to the formation of type B tumors, *KRAS* mutations may not be essential for it. Moreover, the result raises another possibility that tumors harboring *KRAS* mutations may not show type B formation, that is, the alveolar structure collapsing, or may sequentially and rapidly progress from type A to type C featuring an invasive phenotype due to acquisition of additional genetic alterations. To verify this assumption, further analyses of a larger number of those subtypes are needed.

We also examined a total of 124 cases used in this study for mutations in exon 20 of the *ErbB2* gene, a member of the *EGFR* family. The mutation was detected only in 1 tumor (data not shown). This tumor was 5 cm in size and the patient with this tumor was male and a smoker. This *ErbB2* mutation was a 12-bp in-frame duplication/insertion coding for the amino acids TVMA at codon 776. It was recently reported that *ErbB2* mutations were present in 1–4% of lung cancers and that *EGFR*, *ErbB2* and *KRAS* mutations were never present together in individual tumors.[25,26] In the present study, the tumor with *ErbB2* mutation also had no *EGFR* and *KRAS* mutations. As a whole, in the first series of analysis, 49 of the 107 stage I lung adenocarcinomas (46%) had either *EGFR*, *ErbB2* or *KRAS* mutations, suggesting that mutations in those 3 genes contribute to the development of about half of the stage I lung adenocarcinomas.

One of the recent strategies of cancer therapy is to discover mutated oncogenes, which play key roles in tumor growth and progression, and to develop drugs targeted to their protein prod-

TABLE IV – TYPES OF *EGFR* MUTATIONS IN STAGE I LUNG ADENOCARCINOMA

Type of mutation	Exon	Nucleotide number and sequence	Amino acid change	Case with mutation (%)
In-frame deletion	19	2235–2249 del GGAATTAAGAGAAGC	E746–A750 del	14 (26)
	19	2236–2250 del GAATTAAGAGAAGCA	E746–A750 del	10 (19)
	19	2236–2244 del GAATTAAGA, 2245–2248 GAAG>AGCC	E746–R748 del, EA749–750SP	1 (2)
	19	2237–2248 del AATTAAGAGAAG, 2249–2253 CAACA>TTGCT	E746–E749 del, AT750–751VA	1 (2)
	19	2240–2254 del TAAGAGAAGCAACAT	L747–T751 del	1 (2)
	19	2240–2257 del TAAGAGAAGCAACATCTC	L747–S752 del, P753S	1 (2)
In-frame duplication/ insertion	20	2308–2316 ins GCCAGCGTG	ASV 770–772 ins	2 (4)
	20	2311–2319 ins AGCGTGAAC	SVD 771–773 ins	1 (2)
	20	2327–2338 ins CCTACGTGTGCC, 2328 C>T	PYVC 776–779 ins	1 (2)
Point mutation	18	2125 G>A	E709K	1 (2)
	18	2126 A>G	E709G	2 (4)
	20	2303 G>T	S768I	2 (4)
	20	2369 C>T	T790M	1 (2)
	20	2405 T>G	V802G	1 (2)
	21	2573 T>G	L858R	13 (25)
Total				*52 (100)*

ucts.[27] In lung cancer, NSCLC with *EGFR* mutations was revealed to be sensitive to treatment with gefitinib, a TK inhibitor of EGFR. However, the role of this drug in therapeutic strategies of NSCLC has not yet been established. At present, gefitinib is generally applied to pretreated advanced NSCLC as a second or third line of chemotherapy. However, by considering our results that *EGFR* mutations were present in a majority of BACs, this drug can be used for earlier stage disease rather than advanced stage disease. This suggestion is supported by Dowell and Minna.[28] They indicated the possibility to use relatively nontoxic TK inhibitors as chemopreventive agents, if *EGFR* mutations are present in preneoplastic lesions.

In addition, recent studies revealed that the threonine to methionine mutation at codon 790 (T790M) of the *EGFR* gene was a second mutation and was associated with acquired resistance to EGFR TK inhibitors in NSCLC.[29,30] In these reports, this mutation was found in specimens from the patients whose disease progressed during the treatment with these drugs, and not found in untreated cases. Thereafter, Toyooka *et al.*[4,31] identified the T790M mutations in addition to L858R mutations in tumors before chemotherapy or radiotherapy in 2 NSCLC patients, both of whom later had recurrent diseases and eventually died. Therefore, they suggested that tumors with these double mutations are very aggressive. In the present study, we also detected the T790M mutation with another mutation (deletion from codon 747 to 751 in exon 19) in a patient who had received no chemotherapy or radiotherapy before undergoing surgical resection and died due to disease recurrence 50 months after surgery. This case showed similar unfavorable prognosis to those reported by Toyooka *et al.* and support their suggestion. Thus, detection of the T790M mutation in addition to other types of *EGFR* mutations is also important to establish the role of EGFR TK inhibitors in future therapeutic strategies against lung adenocarcinoma and to evaluate the aggressiveness of lung adenocarcinoma.

In summary, frequent *EGFR* mutations in types A and B of small-sized lung adenocarcinoma strongly indicated that the mutations are a critical genetic alteration for the formation of noninvasive BACs. The present study was performed using stage I lung adenocarcinomas, all of which were obtained from Japanese patients. Given the more frequent occurrence of *EGFR* mutations in the Japanese than in the Caucasians, type A and B tumors, which correspond to BAC in the WHO classification, may be a relatively specific subtype of lung adenocarcinoma in the Japanese.

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Radiologic Classification of Small Adenocarcinoma of the Lung: Radiologic-Pathologic Correlation and Its Prognostic Impact

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Background. A new radiologic classification for small adenocarcinoma is necessary for discussions of limited surgical resection for peripheral lung cancer.

Methods. Between 1999 and 2003, 1,697 consecutive patients underwent pulmonary resection for lung cancer. Three hundred forty-nine of these patients with clinical stage IA lung cancer who had lung peripheral adenocarcinoma, 2 cm or less in size, were investigated retrospectively. Radiologic classification was based on the findings of thin-section computed tomographic scan such as the presence of solid and ground-glass opacity (GGO). Type 1 (n = 22), type 2 (n = 26), type 3 (n = 25), and type 4 (n = 43) show a simple GGO, an intermediate homogeneous increase in density, a halo, and a mixed area of GGO and a solid, respectively. Type 5 (n = 54) shows a solid tumor with GGO, and type 6 (n = 179) shows a solid tumor.

Results. There was no difference in the maximum tumor dimension among the six groups. All but 1 patient had no lymph node metastases among type 1 to 4 tumors, whereas these were found in 5% and 24% of the patients with type 5 and 6 tumors, respectively. Lymphatic invasions were rarely found in patients with type 1 to 4 tumors ($p < 0.001$).

Conclusions. Types 1, 2, 3, and 4 are considered to be radiologic early adenocarcinoma of the lung, and their pathologic features were minimally invasive. On the other hand, type 5 and 6 tumors could have lymph node metastases and are considered to be invasive adenocarcinoma. Although limited surgical resection may be enough for type 1 to 4 tumors, anatomic pulmonary resection should be recommended for type 5 or 6 tumor.

(Ann Thorac Surg 2006;81:413–20)

Several authors have reported that the incidence of adenocarcinoma of the lung has been increasing [1, 2]. The introduction of computed tomography (CT) for screening of lung cancer has made it possible to detect smaller pulmonary nodules. Most of those pulmonary nodules are peripherally located adenocarcinoma of the lung, and such early detection may be associated with attainment of cure through early intervention [3, 4]. Although there is a general consensus regarding the pathologic diagnosis of early adenocarcinoma of the lung [5–8], the clinical and radiologic diagnosis of early adenocarcinoma with favorable prognosis is still controversial. Several authors have reported that adenocarcinoma of the lung that shows a wide area of ground-glass opacity (GGO) has a good prognosis [4, 9–15]. However, there is no generally accepted method for measuring the area of GGO, as it is sometimes difficult to divide peripherally located adenocarcinomas according to the existing classification. Thus, a new classification of peripherally located adenocarcinoma of the lung is necessary, and in this study we sought to determine how to best classify peripherally located adenocarcinoma of the lung retrospectively.

Accepted for publication July 18, 2005.

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Patients and Methods

Patient Characteristics

Between January 1999 and December 2003, 1,697 consecutive patients underwent pulmonary resection for lung cancer. Among them, 349 patients with clinical stage IA lung cancer who had peripherally located adenocarcinoma of the lung 2 cm or less in size were investigated in this study. Patients who received preoperative treatment, such as radiotherapy or chemotherapy, or who had multiple lung cancers were excluded from the study. Informed consent was obtained from the patients. Of these, 167 were men and 182 were women. Their ages ranged from 23 to 89 years, with a median of 64 years.

Radiologic Evaluation

Contrast-enhanced CT scan was performed using a TCT 900S or X-Vigor (Toshiba, Tokyo, Japan), and 10-mm-thick contiguous collimation was used to evaluate the entire lung for preoperative staging. The size of tumors was determined digitally based on the findings of thin-section CT

Published by Elsevier Inc

0003-4975/06/$32.00
doi:10.1016/j.athoracsur.2005.07.058

Table 1. Radiologic Classification of Small Adenocarcinoma of Lung by Means of Thoracic Thin-Section Computed Tomography

Class	Radiologic Findings
Type 1	Pure (simple) GGO
Type 2	Semiconsolidation (an area of intermediate homogeneous increase in density)
Type 3	Halo (area consisting of solid part and surrounding GGO halo)
Type 4	Mixed (an area consisting of GGO and solid part having air-bronchogram)
Type 5	Solid pattern with GGO[a]
Type 6	Solid pattern

[a] The area of GGO should be less than 50%.

GGO = ground-glass opacity.

scan. We perform thin-section cuts for every lung tumor 2.0 cm or less in maximal dimension. All tumors were subsequently evaluated with thin-section CT scan. Helical scans with 2-mm collimation were performed through a primary tumor. Images were reconstructed with a high-frequency algorithm, and photographed with a window level of −600 H and a window width of 2,000 H, as a "lung window." Radiologic findings were evaluated by two observers (M.K. and K.S.), who were not informed of the pathologic and prognostic outcome, on thin-section CT scan.

Radiologic Criteria for Grouping

The radiologic findings evaluated were as follows: the maximal tumor dimension, the presence and extent of solid or GGO component in tumor, and homogeneity of tumor. The solid (or consolidation) component was defined as an area of increased opacification more than 5 mm in diameter, which completely obscured underlying vascular markings. Ground-glass opacity was defined as an area of a slight, homogeneous increase in density, which did not obscure underlying vascular markings. Semiconsolidation was defined as an area of an intermediate homogeneous increase in density, which did not obscure underlying vascular markings. A halo was an area that consisted of a solid part and a surrounding GGO halo. Mixed was an area with a heterogeneous increase in density, which consisted of GGO and a solid part with an air-bronchogram. We divided the 373 small adenocarcinomas of the lung into six groups based on the extent of the solid component, presence of GGO, and homogeneity of the tumors (Table 1, Fig 1). Type 1 and 2 tumors are homogeneous in density, and lack a solid component (Figs 2, 3). The density of the tumor distinguishes type 1 from type 2. Type 3 and 4 tumors are heterogeneous in density, and the solid component comprises less than 50% of its diameter. The patterns of the solid component and GGO distinguish type 3 from type 4 (Figs 4, 5). Type 5 and 6 tumors are those that predominantly have a solid component. The presence of GGO distinguishes type 5 from type 6 (Figs 6, 7).

Clinicopathologic Factors and Statistical Consideration

The medical record of each patient was examined for age, sex, histologic tumor type, mode of surgery, serum carcinoembryonic antigen (continuous variable; nanograms per milliliter), pathologic nodal status, lymphatic invasion, vascular invasion, pleural invasion, and intrapulmonary metastasis. Skip metastasis was defined as any mediastinal lymph node involvement by lung cancer without N1 disease. The relationships between these pathologic factors and radiologic classification were investigated in this study to elucidate the prognostic significance of our radiologic classification of peripherally located adenocarcinoma of the lung. To compare two factors, Fisher's exact test was used for statistical analysis. Univariate and multivariate analyses were used to determine which clinical factors predict nodal involvement, such as N1 disease or skip metastasis. Univariate and multivariate analyses were performed by logistic regression analysis using StatView 5.0 (SAS Institute, Inc, Cary, NC). Forward and backward stepwise procedures

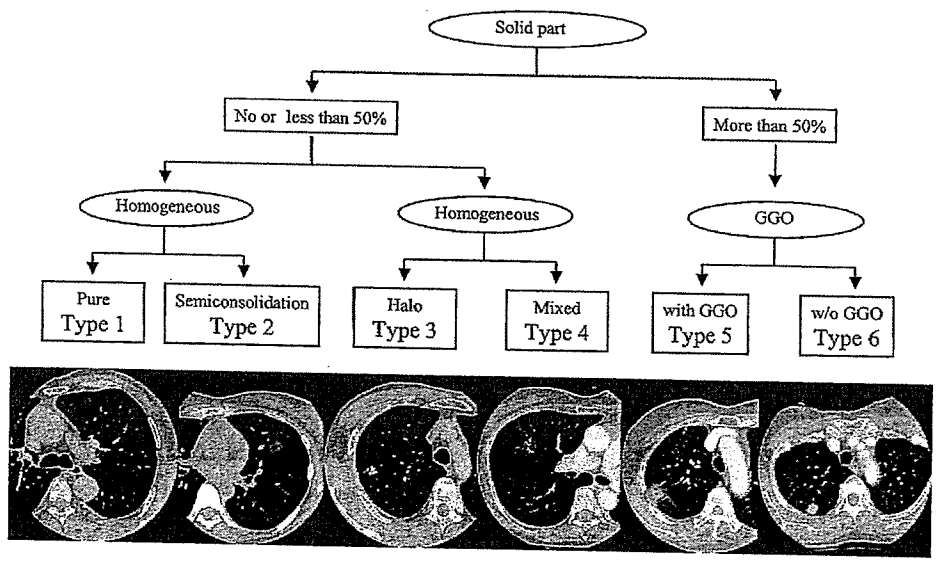

Fig 1. Flow chart for the new classification of small adenocarcinoma of the lung. (GGO = ground-glass opacity; w/o = without.)

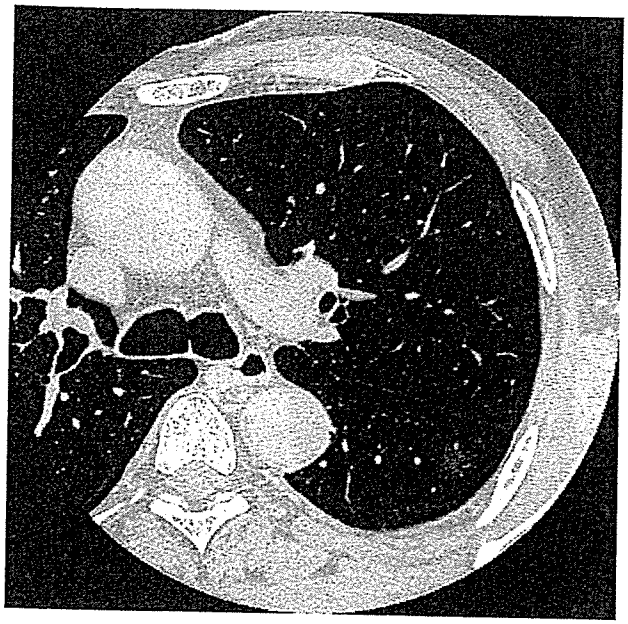

Fig 2. Type 1 tumor is homogeneous in density, and this tumor has been called "pure GGO" or "simple GGO." (GGO = ground-glass opacity.)

were used to determine the combination of factors that were essential in predicting prognosis. Statistical analysis was considered to be significant when the probability value was less than 0.05. Although survival data are shown in this study, this information is considered to be merely suggestive because of the short median follow-up period (just 30 months) for the 341 surviving active patients.

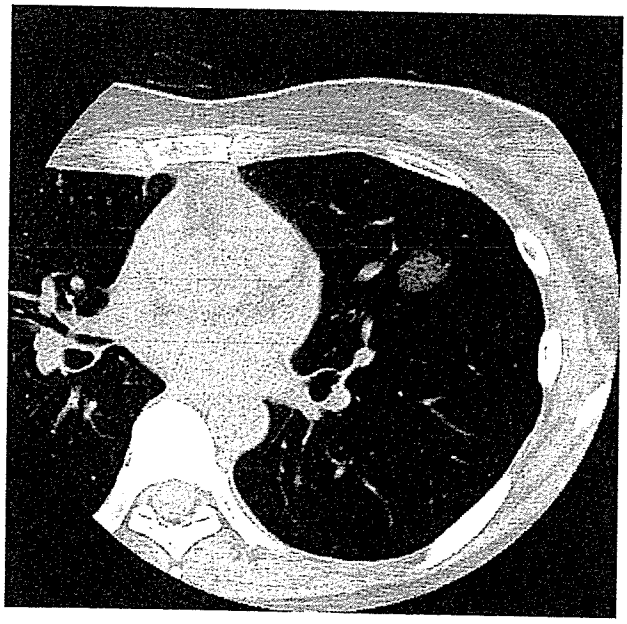

Fig 3. Type 2 tumor is homogeneous in density. It is too dense to call it "pure GGO." The density is much denser than type 1 tumor. (GGO = ground-glass opacity.)

Fig 4. Type 3 tumor is heterogeneous in density, and the solid component comprises less than 50% of its diameter, and is composed of solid and surrounding GGO. (GGO = ground-glass opacity.)

Results

Clinical Characteristics by Radiologic Classifications

Patients with resected adenocarcinoma of the lung 2 cm or less in size were divided into six groups (Table 2). Type 1, 2, 3, 4, 5, and 6 tumors were found in 22 (5.9%), 26 (7.4%), 25 (7.2%), 43 (12.3%), 54 (15.5%), and 179 (51.3%)

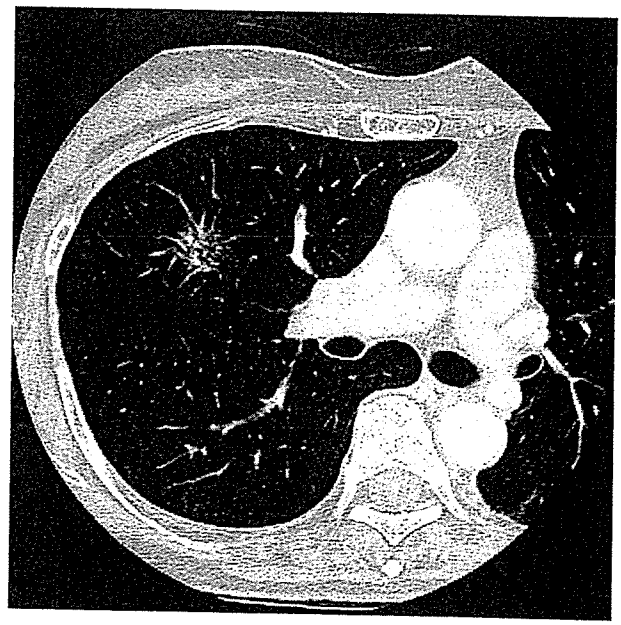

Fig 5. Type 4 tumor is heterogeneous in density, and the patterns of the solid component and GGO distinguish type 3 from type 4. (GGO = ground-glass opacity.)

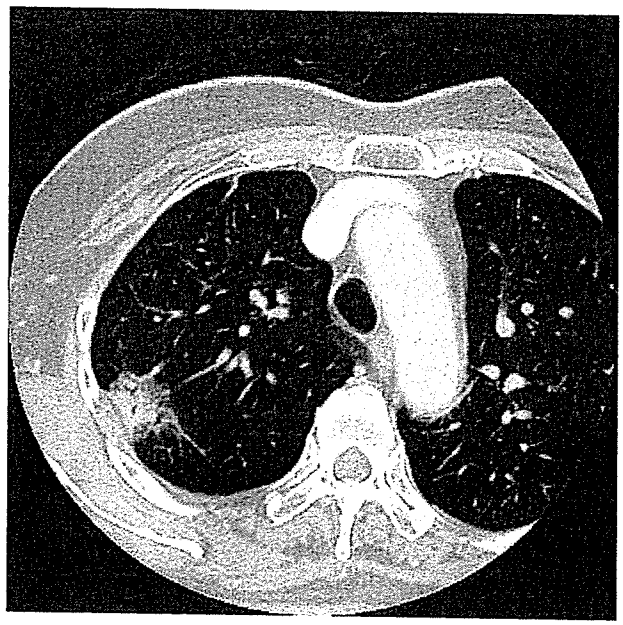

Fig 6. Type 5 tumor predominantly has a solid component and surrounding GGO. (GGO = ground-glass opacity.)

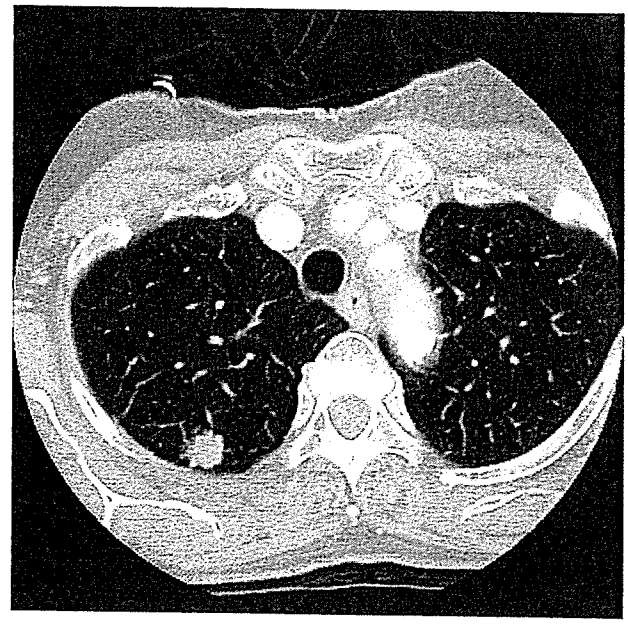

Fig 7. Type 6 tumors predominantly have a solid component. This tumor is so-called pure solid.

patients, respectively. With regard to sex differences, women outnumbered men in each category except type 6. The radiologic maximal tumor dimension ranged from 0.6 to 2.0 cm, with a mean of 1.5 cm, and there were no significant differences among the six categories. Although approximately 20% of patients with type 5 or 6 tumors did not have stage I disease, all but 1 patient with tumors in the other types had stage I disease ($p < 0.001$).

Pathologic Characteristics by the Radiologic Classifications

No nodal involvement was observed among patients with type 1, 2, or 4 tumors. One (4%) patient in type 3 had N1 disease, and 3 patients (5.6%) with type 5 tumors had nodal disease; one N1 and two N2. Type 6 tumors frequently metastasized to regional lymph nodes (43 [24%] patients). Lymphatic invasion was rarely found in patients with type 1, 2, 3, or 4 tumors, whereas this was frequently found in patients with type 5 or 6 tumors ($p < 0.001$). Similar findings were observed for vascular and pleural invasion (Table 3). There were 7 overall deaths, and all died of cancer. All of these patients had lung adenocarcinoma, which showed just a solid component on thin-section CT scan, ie, type 6 tumors. There were no deaths in patients in types 1 to 5, although the median follow-up period for surviving patients is just 30 months.

Table 2. Radiologic Classification and Clinicopathologic Features in Adenocarcinoma of the Lung

Variable	Type 1 Pure GGO	Type 2 SC	Type 3 Halo	Type 4 Mixed	Type 5 Solid & GGO	Type 6 Solid
Number of cases	22 (6.3%)	26 (7.4%)	25 (7.2%)	43 (12.3%)	54 (15.5%)	179 (51.3%)
Mean age (y)	58.6	56.4	64.1	62.3	62.9	62.9
Sex (men/women)	9/13	7/19	11/14	19/24	22/32	99/80
CEA > 5 ng/mL	1	0	1	3	3	40
Radiologic tumor size (cm)						
Range	0.6–1.9	0.8–2.0	0.8–1.9	0.9–2.0	0.9–2.0	0.8–2.0
Mean	1.2	1.4	1.5	1.4	1.5	1.5
Pathologic stage						
IA	21	25	24	43	51	130
IB	1	1	0	0	0	5
IIA	0	0	1	0	0	19
IIB	0	0	0	0	0	4
IIIA	0	0	0	0	2	18
IIIB	0	0	0	0	1	3
IV	0	0	0	0	0	0

CEA = carcinoembryonic antigen; GGO = ground-glass opacity; SC = semiconsolidation.

Table 3. Relationship Between Radiologic Classification and Pathologic Characteristics in Resected Adenocarcinoma of the Lung

Variable	Type 1 Pure GGO	Type 2 SC	Type 3 Halo	Type 4 Mixed	Type 5 Solid & GGO	Type 6 Solid
Total cases	22 (6.3%)	26 (7.4%)	25 (7.2%)	43 (12.3%)	54 (15.5%)	179 (51.3%)
pT1/pT2–T4	21/1	26/0	25/0	43/0	53/1	165/14
Lymph node metastasis	0 (0%)	0 (0%)	1 (4%)	0 (0%)	3 (5.6%)	43 (24%)
pN1/pN2	0/0	0/0	1/0	0/0	1/2	23/20
Skip metastasis	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (1.9%)	7 (4.0%)
Lymphatic invasion	1 (5%)	0 (0%)	1 (4%)	0 (0%)	10 (18.5%)	84 (47%)
Vascular invasion	1 (5%)	0 (0%)	0 (0%)	1 (2%)	5 (9.3%)	86 (48%)
Pleural invasion	2 (9%)	0 (0%)	0 (0%)	1 (2%)	4 (7.4%)	48 (27%)

GGO = ground-glass opacity; SC = semiconsolidation.

Clinical Predictors for Nodal Involvement, N1 Disease, and Skip Metastasis

On the basis of multivariate analysis, preoperative carcinoembryonic antigen (nanograms per milliliter; continuous variable) and radiologic findings (types 1 through 4 versus types 5 and 6) were significant predictors for nodal involvement (Table 4). As to N1 disease, preoperative carcinoembryonic antigen (nanograms per milliliter; continuous variable), and radiologic findings (types 1 through 4 versus types 5 and 6) were again significantly associated with pathologic N1 disease (Table 5). None of the clinical factors were detected to be predictors for so-called skip metastasis.

Comment

Recent investigation of small adenocarcinoma of the lung has revealed the pathologic characteristics of these tumors detected by CT scan. Several authors insisted that the prognosis of lung adenocarcinoma with a large area of GGO on thin-section CT scan was much better than that of conventional adenocarcinoma of the lung regardless of the maximal tumor dimension (Table 6) [4, 9–15]. These reports provide interesting material for discussion. If adenocarcinoma with a good prognosis can be diagnosed preoperatively, major lung resection might not be required. Some authors have already adopted segmental resection for small-sized lung cancer, and have reported that it might be acceptable for patients with a tumor of 2.0 cm or less in diameter without nodal involvement [16, 17]. From these reports, a peripherally located lung cancer with no lymph node metastasis is the optimal indication for a more limited anatomic resection. However, it is difficult to determine the pathologic nodal status during surgical resection, and there could be some discrepancy between the results of intraoperative frozen-section diagnosis and the final pathologic diagnosis of lymph node metastasis. Locoregional recurrence has been noted after extended segmental resection, and it is possible that such local recurrence might have been prevented by pulmonary lobectomy. Thus, the preoperative diagnosis of the biologic invasiveness of a lung cancer is crucial whenever surgeons dare to adopt a lesser anatomic resection for a resectable lung cancer, which could raise the question of compromised patients.

According to previous data, lung adenocarcinoma with a large area of GGO shows a good prognosis, and one of the most important prognostic factors is the extent of GGO. However, how can the extent of GGO be evaluated in patients with type 4 adenocarcinoma? Inasmuch as type 4 tumor is made up of a heterogeneous mixture of GGO and a solid part, it is difficult to measure the size of the solid part. As a result, there is considerable disagreement among physicians on the diagnosis. Some may diagnose such tumors as "noninvasive," and others may diagnose them as "invasive" based on CT findings. The reason for this inconsistency is probably that the former radiologic classification is ill-suited for evaluating peripheral lung adenocarcinoma. The extent of GGO is insufficient for the evaluation of all adenocarcinoma of the lung. It may still be difficult for some surgeons to classify small adenocarcinoma of the lung based on our classification.

Among the six types of peripheral small-sized adenocarcinoma, women were predominant in all types except among patients with type 6 tumors. This is an unexpected finding. Traditionally, lung cancer is found more often in men than women. There was no significant difference among the types with regard to the maximal tumor dimension. Regarding small-sized adenocarcinoma of the lung, Noguchi and colleagues [6] investigated prognostic factors based on the findings of central fibrosis. They stated that type A or B tumors should be considered "in-situ" adeno-

Table 4. Results of Multivariate Analysis for Predictors of Nodal Involvement

Variable	Risk Ratio	95% Confidence Interval	*p* Value
CEA (continuous variable; ng/mL)	1.088	1.025–1.155	0.0059
Radiologic classification (type 1–4 vs 5–6)	0.057	0.008–0.427	0.0052

CEA = carcinoembryonic antigen.

Table 5. Results of Multivariate Analysis for Predictors of N1 Disease

Variable	Risk Ratio	95% Confidence Interval	*p* Value
CEA (continuous variable; ng/mL)	1.092	1.029–1.158	0.0037
Radiologic classification (type 1–4 vs 5–6)	0.074	0.010–0.554	0.0112

CEA = carcinoembryonic antigen.

carcinoma of the lung. It is probably safe to say that segmental resection or wide wedge resection is sufficient for such tumors because of their minimally invasive nature. Type 1 tumor is also known as pure GGO or simple GGO [18]. Among 22 type 1 tumors, there was no lymph node metastasis, and pathologic findings showed minimal invasion. There were 15 (68%) tumors that were equivalent to the type A or B tumors of Noguchi and colleagues [6], ie, roughly bronchioloalveolar carcinoma. Type 2 tumor is denser than type 1 tumor on thin-section CT scan. This tumor is not a solid tumor because we can see the underlying bronchovascular structure. No lymph node metastasis was noted, and 11 tumors were similar to the type A or B tumors of Noguchi and colleagues [6]. The difference in their density is probably related to the difference in the amount of air contained in the tumor, ie, differences in alveolar space histologically. Type 3 tumor is also known as GGO halo. One tumor had metastasized to the intrapulmonary lymph node, ie, N1 node, but 15 tumors were still diagnosed as being equivalent to the type A or B tumor of Noguchi and colleagues [6]. Type 4 tumor is actually defined by our original definition. This tumor consists of a mixture of GGO and a solid part containing air, roughly air-bronchogram. There was no lymph node metastasis and no lymphatic invasion. Basically, lung adenocarcinoma in the above four types is thought to be "minimally invasive" adenocarcinoma. A limited anatomic resection of the lung could be the standard surgical procedure for such tumors in the near future.

Type 5 and 6 tumors are considered to exhibit a "solid" course. Lymph node metastasis was found in roughly 5% of type 5 tumors, and 27% of type 6 tumors. Traditionally, lymph node metastasis is found in approximately 15% of small adenocarcinoma 2.0 cm or less in size. According to our results, however, lymph node metastasis was found mostly in type 6, which meant that if peripheral lung adenocarcinoma showed GGO on thin-section CT, the probability of lymph node metastasis was less than 5%. These "solid" tumors could be divided into several subgroups by means of positron emission tomography. If the solid tumors show positive results by positron emission tomography, they may be associated with a high frequency of lymph node metastasis and a poor prognosis.

One of the important objectives of this study is to determine the indication for limited surgical resection for lung adenocarcinomas. From this concept, the classification became simpler if the classification was composed with groups, ie, types 1 through 4 and types 5 and 6. If a tumor belongs to types 1 through 4, the patient would be a candidate for limited surgical resection, whereas a tumor belonging to group 5 or 6 warrants major lung resection with systematic lymph node dissection necessary. However, we believe the six classifications proposed in this study remain important for the surgeon to plan for the management of peripheral lung cancer. For instance, most of the type 1 tumors are bronchioloalveolar carcinoma, and some of them might be indolent tumors. On the contrary, type 2 tumors tend to be adenocarcinoma with invasive foci pathologically and grow in size. Actually we made a plan for a prospective follow-up study for type 1 tumors, not for type 2 tumors. Thus, clinical strategy depends on the six classifications, and we hope to leave the classification intact.

As to the surgical indications for pure GGO tumors, we resected the tumor if it is stable or increased in size. However, from our data, tumors belonging to type 1 could be bronchioloalveolar carcinoma, and are sometimes indolent. Thus, recently we just monitor such type 1 tumors without surgical interventions if the radiologic maximal

Table 6. Review of Literature Regarding Proportion of Ground-Glass Opacity as Radiologic Prognostic Factors in Adenocarcinoma of the Lung

Authors	Year	No.	Cases	Methods	Good Prognosis	Analysis
Jang et al. [9]	1996	14	...	...	Focal area of GGO	Univariate
Aoki et al. [4]	2001	127	Ad, cT1	Dimension	GGO > 0.5	Univariate
Kodama et al. [10]	2001	104	Ad, 2 cm or less	Visual	GGO > 0.5	Multivariate
Takamochi et al. [19]	2001	269	Ad, peripheral	TDR	TDR & CEA	Multivariate
Kim et al. [11]	2001	224	Ad, cT1	Visual	GGO extent	Univariate
Matsuguma et al. [12]	2002	111	Ad, cIA	Visual	GGO > 0.5	Univariate
Takashima et al. [15]	2002	64	Ad, 2 cm or less	CT	GGO > 0.57	Multivariate
Suzuki et al. [14]	2002	69	Ad, cIA	Dimension	GGO > 50%	Univariate
Okada et al. [17]	2003	167	Ad, cT1	TDR	TDR > 0.5	Multivariate
Ohde et al. [13]	2003	98	Ad, cT1	Dimension	GGO > 50%	Univariate

Ad = adenocarcinoma; CEA = carcinoembryonic antigen; cT1 = clinical T1; GGO = ground-glass opacities; TDR = tumor disappearance ratio.

tumor dimension is less than 15 mm. If radiologic findings suggest the tumor as lung cancer, preoperative CT-guided fine-needle biopsies are not always performed because of the high rate of a false-negative result for GGO tumors.

In conclusion, a new radiologic classification of small-sized adenocarcinoma of the lung has been proposed. Because this is the retrospective study, there may be numerous levels of bias. Therefore, we are planning to perform a prospective study of the management of peripheral small adenocarcinoma of the lung. Using the classification, we can easily classify peripheral adenocarcinoma of the lung into six categories, and the classification is significantly associated with pathologic prognostic factors. Future treatment strategies for small-sized adenocarcinoma of the lung may be based on this new radiologic classification.

The authors thank Dr Etsuo Miyaoka, PhD, a professor of Tokyo University of Science, for his technical support for statistical analysis. This work was supported in part by a Grant-in-Aid for Cancer Research from the Ministry of Health and Welfare.

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INVITED COMMENTARY

Anatomical resection is the standard treatment for early stage nonsmall cell lung cancer. As radiographic scanning methods are improving and we are identifying cancers at smaller sizes than previously recognized, the issue of limited resection for small peripheral cancers is being reinvestigated. Should small size on computed tomography (CT) be the only criteria with which to determine the type of resection (ie, anatomical versus limited [wedge]) to be performed? The answer would be no, according to the article by Suzuki and colleagues **[1]**. This study is a retrospective review of a single institutional experience in 349 chemotherapy–radiotherapy naive patients with small, single peripheral lung primary adenocarcinomas during a 4-year period of time from 1999 to 2003 to evaluate a new radiographic classification that may assist in the future management of patients. From their classification, in essence a radiologic Noguchi classification **[2]**, they were able to identify a group of patients who might be best treated with limited resection. In their series, the 42 patients with either N1 or N2 disease seemed to have a greater solid component and less ground glass opacification (GGO) features than those who did not have those features. The authors concluded that their classification may be a useful evaluation system for future trials.

Some articles raise more questions than answers, as does this article. Clinicians should not be tempted to follow the authors' implications (given the retrospective design of this study) that thin-section CT peripheral lung

0003-4975/06/$32.00
doi:10.1016/j.athoracsur.2005.09.040

Ann Thorac Surg
2006;82:1508–10

Fig 3. (Left) After the lung was deflated, a 15-gauge needle electrode was introduced under ultrasound guidance and was stabilized by hand. (Right) Wedge resection for the tumor was performed using an endo-stapler after radiofrequency ablation.

minutes. The tumor was then resected with a 2-cm margin using a stapler; however, the margin on the central side of the tumor was not sufficient because of the organization and hyalinization of the lung due to prior radiation. The ablated lung tissue was reinforced by mattress sutures with absorbable felt. The total operation time was 144 minutes, and bleeding volume was less than 10 mL. The tumor was diagnosed as a second recurrent squamous cell carcinoma. The postoperative course was uneventful, and the patient was discharged home without complaints. Eighteen months after surgery the patient is alive without recurrence.

Comment

Recently the opportunity for limited surgery for early, recurrent, or metastatic lung cancer has been increasing. However, in this surgical mode, narrow resection margins remain a concern. Usually limited surgery is performed to maintain a macroscopically safe margin, and frozen-section histologic examination is routinely performed. However, the resected line of limited surgery cannot always be sufficiently wide owing to anatomic, physiologic, or technical reasons, especially when the patient has comorbid conditions [3, 4]. Shennib and colleagues [3] reported that only 46% of patients undergoing limited resection had a surgically wide (>1 cm) resection margin. Moreover, Martini and colleagues [1] reported that 31 of 62 patients (50%) who had wedge resection or segmentectomy had recurrence despite complete resection. To decrease the local recurrence rate, Higashiyama and colleagues [4] reported a lavage cytologic technique for the surgical margin, and Santos and colleagues [5] reported intraoperative brachytherapy after sublober resection in high-risk lung cancer patients.

Recently SRT has been introduced as a new modality that allows the delivery of higher doses of radiation to the targeted tumor [6]. This patient had previously received SRT with good control; however, the new lesion was relatively close to the irradiated area and SRT was not used this time.

Generally RFA is most suitable for tumors smaller than 4 cm and for peripheral nodules. The reported local regression rate for lung cancer is 8.6% to 38% [2]. Radiofrequency ablation has the benefit of low morbidity and low cost, and it is a mobile system that can be repeated; moreover it can be easily used in the operation theater with only 10 to 20 minutes of additional time. Consequently, limited resection with RFA is a novel minimally invasive method for local control, especially in a compromised host. Further studies are necessary to evaluate the local control rate of this novel method.

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Multiple Lung Adenocarcinomas Showing Ground-Glass Opacities on Thoracic Computed Tomography

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It is difficult to distinguish multiple primary lung cancers from pulmonary metastasis. We experienced a case of

Accepted for publication Jan 23, 2006.

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0003-4975/06/$32.00
doi:10.1016/j.athoracsur.2006.01.100

surgically resected lung tumors that showed multiple ground-glass opacities on thoracic computed tomographic scan. There were eight nonsolid and two part-solid ground-glass opacities in the bilateral lungs. Surgical resection was performed because all tumors had a ground-glass opacity appearance on computed tomographic scan, which is compatible with a finding of primary lung adenocarcinoma. The postoperative pathologic diagnoses were two cases of invasive adenocarcinoma, six cases of bronchioloalveolar carcinoma, and eight cases of atypical adenomatous hyperplasia. The patient remains alive without any evidence of recurrence 40 months after surgery. A ground-glass opacity appearance on computed tomographic scan could be interpreted as supportive evidence for multiple primary lung adenocarcinoma rather than pulmonary metastases.

(Ann Thorac Surg 2006;82:1508–10)

Introduction of computed tomography into clinical practice led to an increased number of tiny lung nodules that could be detected on computed tomographic (CT) scans. Generally speaking, it is quite difficult to preoperatively distinguish between multiple lung cancers and intrapulmonary metastasis when the histologic diagnosis is the same. We experienced a lung cancer patient who had multiple ground-glass opacities (GGOs) and underwent surgical resection for these lesions. Postoperative pathologic diagnosis revealed the lesions as multiple adenocarcinomas of the lung. We considered that GGO appearance on preoperative CT scan could be interpreted as a sign of multiple lung adenocarcinomas instead of intrapulmonary metastasis.

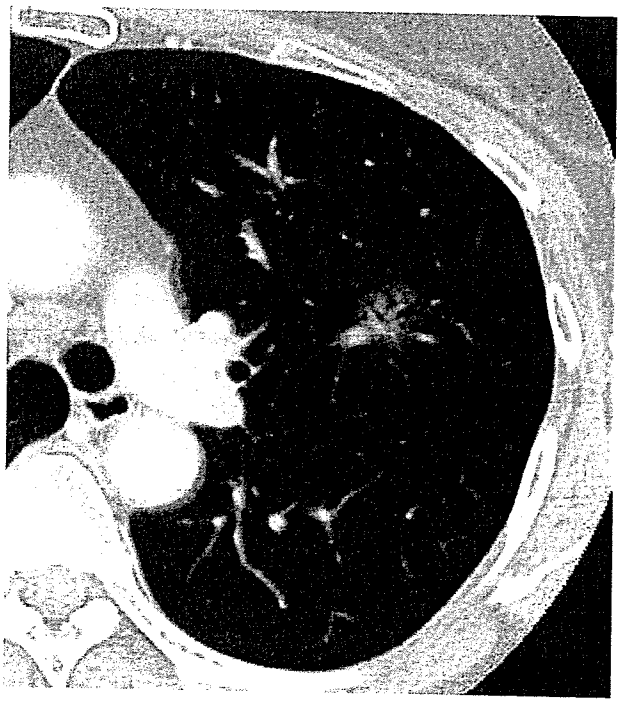

Fig 1. Adenocarcinoma of the lung in the left upper lobe showing ground-glass opacity.

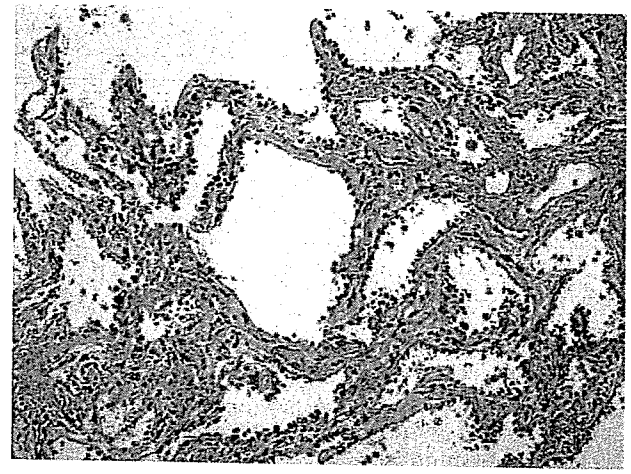

Fig 2. Bronocioloalveolar carcinoma (Hematoxylin and eosin, ×100).

A 60-year-old woman without a remarkable medical history was admitted to our hospital for an evaluation of lung nodules detected by a screening with a CT scan. She had no smoking history and no family history of any cancers. There were multiple GGOs on the thoracic CT scan in the bilateral lungs (Fig 1). Neither hilar nor mediastinal lymphadenopathy was demonstrated. The serum carcino-embroyonic antigen was within normal limits. There were three lung nodules in the right lung on CT scan; one part solid GGO with a maximum dimension of 1.5 cm in the upper lobe, and one GGO each in the middle and lower lobes, respectively. There were five lung nodules in the left lung; one part solid GGO (1.5 cm in diameter), one GGO in the upper lobe, and three GGOs in the lower lobe. An open-lung biopsy was performed from the right side. A right superior segmentectomy and wide wedge resection of the middle and lower lobes with mediastinal lymph node sampling were performed, resulting in four resected lung nodules. A histopathologic examination revealed multiple lung tumors showing extensive bronchioloalveolar spread, which is a feature of primary neoplasm of peripheral airway epithelium. The four tumors consisted of one well-differentiated invasive adenocarcinoma, two bronchioloalveolar carcinomas in the lower lobe (Fig 2), and one atypical adenomatous hyperplasia (AAH) in the middle lobe (Table 1). No tumor cells were found in the lymph nodes. Thus the pathologic diagnosis was right synchronous triple lung cancers of pT1N0M0 accompanied by one AAH. The postoperative course was uneventful. We performed a second operation on the left side 3 months later. Seven wedge resections of the left upper and left lower lobes were performed. A histopathologic examination showed multiple lung tumors that were similar to the right lung. These tumors consisted of one well-differentiated invasive adenocarcinoma and two AAHs in the upper lobe, and four bron-

Table 1. Lobar Distribution of Multiple Lung Cancers and Precancerous Lesions

Location	Histologic Diagnosis		
	Adenocarcinoma	Bronchioloalveolar Carcinoma	Atypical Adenomatous Hyperplasia
RUL	1	0	0
RML	0	0	1
RLL	0	2	0
LUL	1	0	2
LLL	0	4	5

LLL = left lower lobe; LUL = left upper lobe; RLL = right lower lobe; RML = right middle lobe; RUL = right upper lobe.

chioloalveolar carcinomas and five AAHs in the lower lobe (Table 1). No lymph node metastasis was found. The diagnosis was left synchronous five primary lung cancers of pathologic stage T1N0M0 and seven AAHs. The postoperative course was uneventful and no evidence of recurrence has been observed for 40 months.

Comment

It is often difficult to distinguish between multiple primary lung cancers and intrapulmonary metastasis when we encounter patients with multiple lung nodules by radiology. However the diagnosis is quite critical for deciding on the clinical strategy for lung cancers. We encountered a case of multiple lung tumors that showed multiple GGO on thoracic CT scan. Radiographic findings strongly suggested that multiple lung nodules in the present case were multiple primary adenocarcinomas, which were confirmed by histopathology. After multiple lung resections for these tumors, the patient remains alive without evidence of disease. This case suggests that multiple lung nodules displaying GGO on CT are important for differentiating multiple lung cancers from intrapulmonary metastases.

Recent advances in high-resolution CT scanning have resulted in the more frequent detection of GGO. There is still some controversy regarding how to best manage this lesion. Careful follow-up may be sufficient instead of conventional surgical resection. As for the type of surgery, major lung resection such as pneumonectomy or lobectomy is still the standard approach for primary lung cancer. However, limited resection may be suitable for lung cancer detected by CT, because these lesions tend to be early adenocarcinoma [1]. In this case we basically diagnosed the bilateral lesions as synchronous primary adenocarcinomas based on the criteria of Martini and Melamed [2]. Most of the lesions were accompanied by GGO on thoracic CT scan, and we suspected them to be early lung cancers. As a result, multiple limited surgical resections were performed with curative intent.

There are still various controversies regarding the carcinogenesis of lung cancer. There have been some reports on the adenoma-carcinoma sequence in the lung, and AAH, which is believed to be a precursor lesion, is concomitant with lung adenocarcinoma in more than 20% of the cases [3–5]. Although concomitant AAH has no impact on the prognosis, metachronous multiple lung cancers may be found in this population. Thus intensive follow-up is necessary for this situation. In our case, intensive follow-up with thoracic CT scan was indicated for 40 months. So far there have been no new lesions on thoracic CT scan. Further investigations of multiple lung cancers with precancerous lesions are needed.

This work was supported in part by a Grant-in-Aid for Cancer Research from the Ministry of Health, Labor, and Welfare.

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How Fast Does an Atrial Myxoma Grow?

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We describe the case of a 58-year-old man who underwent coronary artery bypass grafting with an unremark-

Accepted for publication Nov 7, 2005.

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0003-4975/06/$32.00
doi:10.1016/j.athoracsur.2005.11.014

肺・胸膜疾患

Mesotheliomaの胸腔鏡所見

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月刊 臨牀と研究 別冊

平成18年6月発行

第83巻 第6号