TABLE 1. Patient Characteristics

Number of Patients	Acquired Resistance (n = 20)	Intrinsic Resistance (n = 44)	Sensitive (n = 29)	Total (n = 93)
Age				
Median	59.5	65.5	65	64
Range	32–85	34–76	42–86	32–86
Gender				
Male	6	26	10	42
Female	14	18	19	51
Smoking history				
Former/current Smoker	3	21	11	35
Never smoker	17	23	18	58
Histological type				
Adeno	19	39	29	87
Large cell	0	1	0	1
Squamous cell	0	2	0	2
Undifferentiated non-small cell carcinoma, or adenosquamous	1	2	0	3
EGFR-TKI treatment				
Gefitinib	19	36	27	82
Erlotinib	1	7	2	10
Vandetanib	0	1	0	1
Number of Tumors	n = 23	n = 45	n = 29	n = 97
EGFR mutation status				
Exon 19 deletion	12	14[a]	14[a]	40
L858R	11	30	16	57
G719X	0	2	0	2

[a] One patient's tumor had both exon 19 deletion and L858R point mutation.

absence of staining; 1+, weaker staining than normal bronchial epithelium; 2+, similar staining to normal bronchial epithelium; and 3+, clearly more intense staining than normal bronchial epithelium) (Supplementary Figure 1, http://links.lww.com/JTO/A197). The percentage and intensity were multiplied to give a scoring index (H score) ranging from 0 to 300, according to a previously reported method with minor modifications.[16] Turke et al.[16] reported that HGF expression was significantly higher in specimens with acquired resistance (mean ± SD: 205 ± 106) compared with pretreatment (126 ± 112). On additional evaluation with specimens showing acquired resistance from patients whose tumors were obtained only after acquiring EGFR-TKI resistance, HGF expression was similar (176 ± 126) to that of specimens with acquired resistance in patients with paired tumor specimens; they concluded that these findings with clinical specimens supported the suggestion that HGF mediated resistance to EGFR-TKIs. Therefore, we defined high-level HGF expression as H score ≥200 in this study. Evaluation was performed independently by two investigators (KT and MN) blinded to individual clinical information.

Cycleave Real-Time Polymerase Chain Reaction Assay for T790M Mutation

Details of the cycleave real-time polymerase chain reaction (PCR) assay have been described previously.[21] Briefly, tumor cell-rich areas in hematoxylin and eosin-stained sections were marked under a microscope, and tissues were scratched from the area of another deparaffinized unstained section. Pieces of the scratched tissue were incubated with 1× PCR buffer containing 100 μg/mL proteinase K for 1 hour at 54°C. After heat inactivation at 95°C for 3 minutes, the solution was used directly as the template DNA for the assay. Then, exon 20 of the *EGFR* gene was amplified by real-time quantitative PCR assay on a SmartCycler (Cepheid, Sunnyvale, CA) using Cycleave PCR Core kits (TaKaRa Co. Ltd., Ohtsu, Japan) with a T790M-specific cycling probe and a wild-type cycling probe. This assay detected as few as 5% cancer cells with T790M mutation in a background of cells with wild-type T790M in *EGFR*.

MET Amplification

Formalin-fixed, paraffin-embedded tissue sections (4 μm thick) were subjected to dual-color fluorescence in situ hybridization using a MET/CEP7 probe cocktail (Kreatech Diagnostics, Amsterdam, The Netherlands) according to the manufacturer's instructions. Staining was evaluated as reported previously.[22,23]

Statistical Analysis

Statistical significance was determined by Student's t test. All statistical analyses were performed using GraphPad

TABLE 2. Expression of HGF, T790M Secondary Mutation, and *MET* Amplification in EGFR-TKI-Resistant Tumors Obtained from *EGFR* Mutant Lung Cancer Patients

	Acquired Resistance (n = 23)	Intrinsic Resistance (n = 45)	Sensitive (n = 29)
High-level HGF expression	14 (61%)	13[a] (29%)	3[b] (10%)
EGFR T790M secondary mutation	12 (52%)	0	0
MET amplification	2 (9%)	2 (4%)	0

[a] High-level HGF expression was detected in the stroma in two patients.
[b] High-level HGF expression was detected in the stroma in one patient.

Prism Ver. 4.01 (GraphPad Software, Inc., San Diego, CA). All tests were two sided, and $p < 0.05$ was taken to indicate statistical significance.

RESULTS

HGF Expression, T790M Secondary Mutation, and MET Amplification in Tumors with Acquired Resistance

Among 23 tumors with acquired resistance from 20 patients, *EGFR* T790M secondary mutation was detected in 12 tumors (52%) from 11 patients (60%) (Table 2). *MET* amplification was detected in two tumors (9%) from two patients (10%). As HGF is a soluble cytokine, evaluation of HGF is not as simple as that for genetically conferred T790M secondary mutation and *MET* amplification, which can be designated as plus or minus. As described in the Materials and Methods section, we defined high-level HGF expression as *H* score ≥200 in this study. High-level HGF expression was detected in 14 tumors (61%) from 13 patients (60%). In these 14 tumors, HGF was predominantly expressed in cancer cells.

The high HGF expression was simultaneously detected in 6 of 12 tumors positive for T790M secondary mutation (50%) (Table 3, Figure 1). High-level HGF expression was also detected simultaneously in one of two tumors positive for *MET* amplification (50%). These results suggested possible interactions among these three resistance factors, consistent with previous reports.[16,17]

Expression of HGF, T790M Secondary Mutation, and MET Amplification in Tumors with Intrinsic Resistance (Nonresponders)

T790M secondary mutation was not detected in 45 tumors with intrinsic resistance from 44 patients (nonresponders), but *MET* amplification was detected in two tumors (4%) (Table 2). *EGFR* D761Y secondary mutation was detected in two tumors (4%) from one patient[24](Supplementary Table 1, http://links.lww.com/JTO/A197). In contrast, high-level HGF expression in cancer cells was detected in 11 tumors (24%) from 11 patients. In addition, HGF was detected at high levels in stromal cells in two tumors (4%) from two patients (data not shown). In total, high-level HGF expression was detected in 13 tumors with intrinsic resistance (29%). Notably, high-level HGF expression was simultaneously detected in one of two *MET* amplification-positive tumors (50%) (Table 2). These results suggested the involvement of HGF in intrinsic resistance to EGFR-TKIs in *EGFR* mutant lung cancer in Japanese patients.

Expression of HGF, T790M Secondary Mutation, and MET Amplification in Sensitive Tumors

Neither *EGFR* T790M secondary mutation nor *MET* amplification was detected in 29 sensitive tumors from 29 patients. High-level HGF expression was detected in two tumors (7%) (Supplementary Table 2, http://links.lww.com/JTO/A197). High levels of HGF were detected in stromal cells in one tumor (3%). In total, a high level of HGF expression was detected in three sensitive tumors (10%). Thus, although high HGF expression level was detected even in sensitive tumors, the incidence of high HGF expression was much lower in sensitive tumors than in those with acquired or intrinsic resistance. In addition, mean *H* score of HGF in tumors with acquired resistance was significantly higher than that in sensitive tumors ($p < 0.001$, Student's *t* test) (Figure 2). There was no significant difference in mean *H* score of HGF between tumors with intrinsic resistance (nonresponders) and sensitive tumors.

DISCUSSION

Our previous studies[14,25,26] documented HGF-mediated resistance to EGFR-TKIs in *EGFR* mutant lung cancer, which was also confirmed by other groups.[16,27] Here, we demonstrated that a high level of HGF expression was detected most frequently in tumors with intrinsic and acquired resistance to EGFR-TKIs in *EGFR* mutant lung cancer in Japanese patients. Our data indicated that although T790M secondary mutation and *MET* amplification are predominantly responsible for acquired resistance, HGF may be responsible not only for acquired resistance but also for intrinsic resistance to EGFR-TKIs.

The mechanism of intrinsic resistance to EGFR-TKIs is not well understood. To our knowledge, this is the first study with more than 40 clinical specimens indicating the incidence of resistance factors in intrinsic resistance to EGFR-TKIs in *EGFR* mutant lung cancer. Here, we found that a high level of HGF expression was most frequently (29%) detected in tumors with intrinsic resistance, compared with T790M secondary mutation (0%) and *MET* amplification (4%). It is noteworthy that although the high HGF expression level was detected in cancer cells in tumors with acquired resistance, HGF expression was detected in both cancer cells (10/12 tumors) and host stroma cells (2/12 tumors) in tumors with intrinsic resistance (nonresponders). HGF was reported to be produced by not only cancer cells but also stromal cells.[15] Our data clearly indicated that both cancer cells and stromal cells are sources of HGF, which induces intrinsic EGFR-TKI resistance in *EGFR* mutant lung cancer. As HGF-induced resistance could be reversed by anti-HGF antibody and the natural HGF inhibitor NK4,[25,27] highly produced HGF in

TABLE 3. Summary of Tumors with Acquired Resistance

ID	Gender	Histological Type	*EGFR* Mutation Status	Treatment	BOR	PFS	HGF	T790M	*MET* Amplification
KZ-1	M	Ad	Exon 19 del	Erlotinib	PR	254	60	−	+
KZ-2	F	Ad	L858R	Gefitinib	CR	1041	40	−	−
KZ-3	F	Ad	L858R	Gefitinib	PR	366	200	−	−
OK1—1	M	Ad	Exon 19 del	Gefitinib	PR	351	290	−	−
OK1—2							300	−	−
OK4—2	F	Ad	Exon 19 del	Gefitinib	PR	57	210	+	−
TS-1—3	F	Ad	L858R	Gefitinib	PR	180	90	−	−
TS-1—4							280	+	−
SG2	M	Ad	Exon 19 del	Gefitinib	PR	174	150	+	−
SG3	F	Ad	L858R	Gefitinib	SD	368	110	+	−
SG4	F	Ad	L858R	Gefitinib	PR	60	220	−	+
SG6	M	Ad	Exon 19 del	Gefitinib	PR	352	140	+	−
SG8	F	Ad	L858R	Gefitinib	SD	210	90	+	−
SG9	F	Ad	Exon 19 del	Gefitinib	SD	221	200	+	−
SG10	F	Ad	L858R	Gefitinib	CR	210	210	−	−
TB1—2	M	Ad	Exon 19 del	Gefitinib	PR	1770	230	+	−
TB2—2	F	AdSq	Exon 19 del	Gefitinib	PR	300	300	−	−
AC29—1	M	Ad	L858R	Gefitinib	PR	533	250	−	−
AC29—2							270	+	−
AC24	F	Ad	Exon 19 del	Gefitinib	PR	98	170	+	−
AC26	F	Ad	Exon 19 del	Gefitinib	SD	448	180	+	−
AC28	F	Ad	Exon 19 del	Gefitinib	PR	357	200	+	−
AC31	F	Ad	L858R	Gefitinib	PR	894	200	−	−

Ad, adeno; AdSq, adenosquamous; BOR, best overall response.

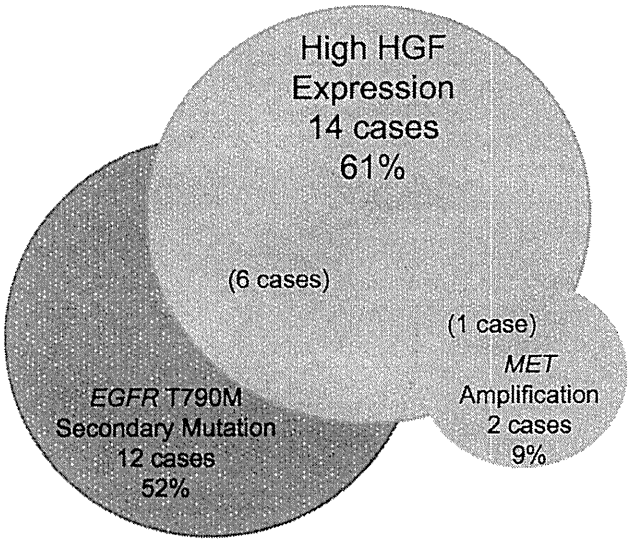

FIGURE 1. Incidences of high-level HGF expression, T790M secondary mutation, and *MET* amplification in 23 tumors with acquired resistance. Values in parentheses are the numbers of cases in which the tumors expressed two resistance factors simultaneously.

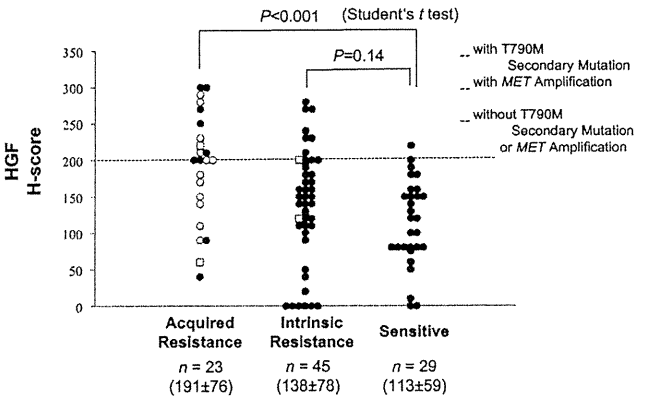

FIGURE 2. HGF expression score (*H* score) in EGFR-TKI-resistant tumors obtained from *EGFR* mutant lung cancer patients. Values in parentheses are mean ± SD of *H* score.

resistant tumors would be an ideal therapeutic target regardless of its origin.

It was of interest that a high level of HGF expression was detected in a small population of sensitive tumors. This was consistent with a previous report[16] indicating high-level HGF expression (*H* score ≥200) in several specimens from responders. Although the reason for the high level of HGF expression in tumors from responders is unclear at present, there are several possible explanations as follows. First, although HGF was expressed at high levels, natural inhibitors such as cleaved HGF and truncated MET, both of which inhibit binding of HGF to MET, may be generated in the tumors.[28,29] Second, negative regulators of MET tyrosine kinase activity such as protein kinase C may be activated and negate the effect of HGF on induction of EGFR-TKI resis-

tance in these tumors.[30] As the amounts of each clinical specimen were limited, we would like to perform further analyses in future studies should sufficient amounts of specimens become available.

Recent studies indicated that multiple resistance factors can be induced simultaneously in a single cancer. For example, Qi et al.[31] reported the simultaneous occurrence of *Met* mutation and activation of the EGFR pathway by ligand overexpression, similar to T790M mutation and HGF overexpression in EGFR mutant lung cancer, which caused resistance to Met-TKIs in gastric cancer. Katayama et al.[32] also reported that *ALK* gene amplification and gatekeeper mutation in *ALK* occurred simultaneously and conferred resistance to ALK inhibitors in EML4-ALK lung cancer. In this study, T790M secondary mutation and the high HGF expression level were simultaneously detected at high incidence (50%) in tumors with acquired resistance. Irreversible EGFR-TKIs were thought to have potential to control acquired resistance caused by T790M secondary mutation, but clinical responses were rarely observed in clinical trials.[33,34] We recently found that HGF induces resistance to not only reversible EGFR-TKIs but also irreversible EGFR-TKIs by activating the MET/PI3K/Akt pathway in *EGFR* mutant lung cancer cells with or without T790M secondary mutation.[26] Taken together, these observations suggest that HGF would be simultaneously expressed with T790M secondary mutation in tumors with acquired resistance and reduce the sensitivity to irreversible EGFR-TKIs in *EGFR* mutant lung cancer patients.

MET amplification has been detected in ~20% of tumors with acquired resistance to EGFR-TKIs in *EGFR* mutant lung cancer,[13,16,17] while the incidence reported in Japanese patients is rare.[14,18] Here, we detected *MET* amplification in two tumors (9%) with acquired resistance, suggesting that *MET* amplification can be detected in a significant proportion of tumors with acquired resistance even in Japanese patients. One case with high-level HGF expression and *MET* amplification (KZ-1) was treated with gefitinib and PFS was 254 days. The other case with low HGF and *MET* amplification (SG4) was treated with erlotinib and PFS was 60 days (Table 3). Although it is not possible to make definitive conclusions based on the data from only these two cases, the shorter PFS in the former case tentatively supports the observation that HGF accelerates expansion of preexisting clones with MET amplification.[16] Notably, simultaneous expression of these two factors was also detected in one tumor with intrinsic resistance (nonresponder). However, the mechanism by which HGF is induced in *EGFR* mutant lung cancer is still not well defined. Further examinations are warranted to elucidate the interaction between HGF expression and *MET* amplification in *EGFR* mutant lung cancer.

Among 68 resistant tumors, high-level HGF expression, T790M secondary mutation, and *MET* amplification were not detected in one tumor with acquired resistance and 31 tumors with intrinsic resistance, indicating the involvement of other mechanisms of resistance in these tumors. *EGFR* D761Y secondary mutation in exon 20 was detected in two tumors from the same patient.[24] *EGFR* D761Y mutation was originally identified in recurrent brain metastasis and was shown to induce intermediate-grade resistance to EGFR-TKIs.[35] In addition, rare secondary mutations (other than T790M and D761Y) or a preexisting resistance mutation in a minority of clones may also be involved in intrinsic resistance. Moreover, it was recently reported that a subpopulation of cancer cells that transiently exhibit a distinct phenotype characterized by engagement of IGF-1R activity, hypersensitivity to HDAC inhibition, and altered chromatin showed an intrinsic ability to tolerate exposure to EGFR-TKI.[36] Minor secondary mutations, a preexisting resistance mutation in a minority of clones, or chromatin-mediated drug resistance mechanisms may be involved in resistant tumors without high HGF expression, T790M secondary mutation, and *MET* amplification.

To overcome the HGF-induced resistance to EGFR-TKI in *EGFR* mutant lung cancer, double blockade of the EGFR pathway and HGF-MET pathway is therefore theoretically necessary.[14,16,27] To inhibit mutant EGFR with or without T790M secondary mutation, EGFR mutant-specific inhibitors were developed in addition to irreversible EGFR-TKIs.[37] To inhibit HGF-MET signaling, several inhibitors, including anti-HGF antibody, NK4 (natural antagonist of MET), and MET-TKIs, were developed.[16,25–27] Further studies are essential to determine optimal combined therapy with best efficacy and safety. In addition, a prospective study is required to determine whether immunohistochemical detection of HGF would be sufficiently reliable to identify patients with HGF-induced resistance to EGFR-TKIs. As levels of HGF in peripheral blood are correlated with clinical outcome to EGFR-TKIs in patients with non-small cell lung cancer,[38,39] such noninvasive methods may facilitate individual therapy for overcoming HGF-induced resistance to EGFR-TKIs in *EGFR* mutant lung cancer patients.

Recent studies indicated at least three important roles of HGF in EGFR-TKI resistance in *EGFR* mutant lung cancer. First, HGF induces resistance to reversible EGFR-TKIs, gefitinib, and erlotinib, by restoring MET/Gab1/PI3K/Akt pathways.[14,16] Second, HGF accelerates expansion of preexisting *MET*-amplified cancer cells and facilitates MET amplification-mediated resistance during EGFR-TKI treatment.[16] Third, after acquiring resistance to reversible EGFR-TKIs, HGF induces resistance of lung cancer cells with T790M secondary mutation to irreversible EGFR-TKIs.[24] Here, we detected high-level HGF expression frequently in tumors with intrinsic and acquired resistance to EGFR-TKIs in *EGFR* mutant lung cancer in Japanese patients. These findings indicate the value of HGF as a therapeutic target for EGFR-TKI-resistant *EGFR* mutant lung cancer. Therefore, combined therapy with EGFR-TKIs and HGF-MET inhibitors in patients with HGF-induced resistance may improve the clinical outcome of *EGFR* mutant lung cancer.

ACKNOWLEDGMENTS

Supported in part by Grant-in-Aid for Cancer Research from the Ministry of Health, Labor, and Welfare (to M.N., 16-1) and from the Ministry of Education, Science, Sports, and Culture of Japan (to S.Y. 21390256, 22112010A01).

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The American Journal of Surgery (2011) 202, 419–426

The American Journal of Surgery®

Clinical Science

Outcome of surgical resection for recurrent pulmonary metastasis from colorectal carcinoma

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KEYWORDS:
Pulmonary metastasis;
Colorectal cancer;
Repeat thoracotomy

Abstract

BACKGROUND: The outcomes after repeat pulmonary resection for colorectal cancer (CRC) and the factors associated with the prognosis of these patients remain uncharacterized.

METHODS: Data on 156 patients who underwent curative resection of pulmonary metastasis from CRC were reviewed. Repeat pulmonary resection was performed in 25 patients; the present study examined the outcomes and factors associated with prognosis after repeat pulmonary resection.

RESULTS: The 5-year survival rate after the first pulmonary resection was 56.2%. A multivariate analysis identified a histological type other than well-differentiated adenocarcinoma, a high prethoracotomy serum carcinoembryonic antigen (CEA) level, and the presence of hilar or mediastinal lymph node metastasis as poor prognostic factors for the first pulmonary resection. The 5-year survival rate after repeat pulmonary resection was 42.1%. Hilar or mediastinal lymph node metastasis at the time of the repeat resection was significantly associated with poor survival.

CONCLUSIONS: Repeat pulmonary resection for metastatic CRC provides satisfactory outcomes. Hilar or mediastinal lymph node involvement is consistently associated with a poor prognosis after the first and repeat pulmonary resections.

Colorectal cancer (CRC) remains one of the leading causes of cancer death in Western countries. More than two thirds of these patients undergo primary curative resection; however, more than half of the resected patients eventually succumb to the disease.[1] The most common sites of recurrence after resection of primary CRC are liver and lung. Patients with untreated metastatic CRCs have a median survival time of less than 10 months and a 5-year survival frequency of less than 5%.[2] Recently, antiangiogenic therapy with bevacizumab combined with oxaliplatin-based chemotherapy was reported to improve the survival time of patients with CRC.[3] However, few patients achieved complete remission using these new treatments, and most patients therefore exhibit disease progression.

Therefore, surgery remains the best treatment for patients with pulmonary metastases from CRC if potentially curative

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Manuscript received July 21, 2009; revised manuscript August 13, 2010

0002-9610/$ - see front matter © 2011 Elsevier Inc. All rights reserved.
doi:10.1016/j.amjsurg.2010.08.016

resection is expected and is an established treatment modality in patients with metastatic CRCs. The reported 5-year survival rates after pulmonary metastasectomy for CRC are 24% to 71.2%.[2–15]

Repeat pulmonary resection is also effective for recurrent pulmonary metastasis.[16] Several authors[5,9,11,13–15,17] have advocated this treatment for patients with metastatic CRCs; however, the outcomes after repeat pulmonary resection for CRC and the factors associated with the prognosis of these patients remain uncharacterized. The present study examined the outcome of surgical resection for recurrent pulmonary metastasis from CRCs and determined the prognostic factors compared with those of the first pulmonary resection.

Patients and Methods

Patient selection

From January 1980 to December 2008, a total of 156 patients with previous CRCs underwent curative pulmonary resection at the Osaka Medical Center for Cancer and Cardiovascular Diseases. Curative resection was defined as follows: no additional extrapulmonary sites of metastatic disease or already resected if present, no locoregional recurrence, and no residual macroscopic tumor tissue after the resection. Histopathological evaluations of the resected lung specimens confirmed CRC metastases in all patients.

Patients were selected for resection of pulmonary metastases after meeting the following criteria: (1) pulmonary metastases were deemed to be completely resectable by preoperative radiological examination, (2) absence of apparent hilar or mediastinal lymph node metastases determined by preoperative radiologic examination, (3) metastatic disease limited to the lungs or extrapulmonary distant metastasis(es) that was controlled or controllable if present, (4) locoregional control of the primary CRC was achieved or achievable, and (5) good general condition and adequate respiratory function to tolerate lung resection.

Before 2006, lymph node involvement was generally assessed by computed tomography (CT) scanning, with lymph nodes diagnosed as positive if they extended more than 10 mm across the short-axis diameter. Since 2006, lymph node involvement is generally assessed by F18-fluorodeoxyglucose positron emission tomography/CT (FDG-PET/CT) in our hospital.

Patient characteristics

Clinical information was obtained from the medical records in our hospital. The median time interval between resection of primary CRC and first pulmonary resection was 27 months (range, 0–109 months). The mean age at the time of first pulmonary resection was 62 years (range, 39–83 years of age). Thirty-eight patients had previously undergone resection for extrapulmonary metastases or local recurrences before the pulmonary resection. Of these, 29 patients underwent liver metastasectomy, 6 patients underwent resection of a local recurrence of the primary tumor, 2 patients underwent inguinal lymph node metastasectomy, and 1 patient underwent resection of a para-aortic lymph node metastasis. Three patients underwent simultaneous resection of metastatic CRCs to the lung and either to an extrathoracic site or local recurrence: thyroid metastasis in 1 patient, brain metastasis in 1 patient, and local recurrence of primary tumor in 1 patient. Perioperative chemotherapy at thoracotomy, including preoperative and/or postoperative adjuvant therapy, was performed in 76 patients as follows: 5-fluorouracil or its derivatives were administered in 61 patients; tegafur in 23 patients; doxifluridine in 21 patients; fluorouracil in 4 patients; carmofur in 3 patients; UFT in 9 patients; and S-1 in 1 patient. Cisplatin-, irinotecan-, and oxaliplatin-based chemotherapy were administered to 6, 4, and 5 patients, respectively. Table 1 summarizes the patient characteristics.

Repeat pulmonary resection

If new nodules had evolved after the first pulmonary resection, a second resection was defined as a repeat pulmonary resection. Planned staged thoracotomy for bilateral metastases was counted as a single operation and was therefore excluded from this study definition. In the survival analysis of patients who underwent a planned staged thoracotomy, the date when the earlier surgery was performed was recognized as the starting point. Repeat pulmonary metastasectomies were also performed if the patient met the criteria for the first pulmonary resection as described earlier. Repeat pulmonary resections were performed in 25 patients; 24 of these patients underwent second pulmonary resections, and 1 patient underwent a third pulmonary resection.

Follow-up schedule

Follow-up generally involved a chest x-ray or a chest and abdominal CT scan, a physical examination, and blood chemistry performed every 6 to 12 months after the first pulmonary resection. Follow-up information was obtained from the medical records in our hospital, letters from the patient's general practitioner, or from the death certificates of the Osaka Cancer Registry. Patients or their families were contacted by phone or by letter if necessary.

Statistical analysis

The statistical analyses were performed using the StatView 5.0 software program (SAS Institute, Berkley, CA). The overall survival after the first and repeat pulmonary resections was analyzed by the Kaplan-Meier method using the dates of the first and second pulmonary resections, respectively, as the starting points. Significance of differ-

Table 1 Patient characteristics and details of the first pulmonary resection

Characteristics	n
Sex	
Male	91
Female	65
Age at the first pulmonary resection (y)	
Mean	62
Range	39–83
Stage of primary tumor	
Dukes A	2
Dukes B	37
Dukes C	85
Dukes D	21
Unknown	11
Location of primary tumor	
Colon	74
Rectum	82
Histology of primary tumor	
Well-differentiated adenocarcinoma	65
Moderately differentiated adenocarcinoma	77
Others*	11
Unknown	3
Interval between primary resection and first pulmonary resection (months)	
Median	27
Range	0–109
Prethoracotomy serum CEA level (ng/mL)	
<5	90
≥5	66
History of surgical treatment of extrathoracic recurrence	
Yes	41
No	115
Repeat pulmonary resection	
Yes	25
No	131
Number of resected metastases	
1	100
2	32
≥3	24
Site of metastasis	
Unilateral	130
Bilateral	26
Maximum tumor size (mm)	
≤30	115
>30	41
Type of resection	
Sublobar resection	99
Lobectomy or pneumonectomy	57
Hilar or mediastinal lymph node metastasis	
Yes	15
No	141

*Others include poorly differentiated adenocarcinoma and mucinous adenocarcinoma.

ences between subgroups was calculated using the log-rank test. The multivariate analysis of prognostic factors was performed using the Cox multivariate proportional hazard model. A P value of less than .05 was considered statistically significant. Data are expressed as the mean ± standard deviation or median values.

Results

Details of first pulmonary resection

There was no operative major morbidity or mortality. Sublobar resection (wide-wedge resection or segmentectomy) was performed in 99 patients, lobectomy in 56, and pneumonectomy in 1 patient. One hundred patients had a solitary metastasis, and 56 patients had multiple metastases. The details of the first pulmonary resections are shown in Table 1. The median time interval between the first pulmonary resection and death or the latest follow-up examination in the present series was 43 months (range, 4–270 months).

Clinical course after the first pulmonary resection

Ninety-three patients developed recurrence after the first pulmonary resection (Fig. 1). The initial pattern of recurrence after lung resection was pulmonary metastasis in 39 patients, including 7 patients with radiologically apparent mediastinal involvement, surgical margin relapse in 5 patients including 1 patient with radiologically apparent mediastinal involvement, pleuritis carcinomatosa in 2 patients, pulmonary metastasis and extrathoracic recurrence in 7 patients, and extrathoracic recurrence in 40 patients. Twenty-five patients underwent a second pulmonary resection; 13 of these subsequently experienced recurrent disease as pulmonary metastasis in 10 patients and extrathoracic recurrence in 3 patients. Only 1 patient underwent a third pulmonary resection. Currently, 69 patients are alive with no evidence of disease, 14 patients are alive with disease, 8 patients died of another disease, and 65 patients succumbed to the disease.

Overall survival of patients after the first pulmonary resection

The cumulative 3-, 5-, and 10-year survival rates after the first pulmonary resection were found to be 71.4%, 56.2%, and 44.0%, respectively (Fig. 2).

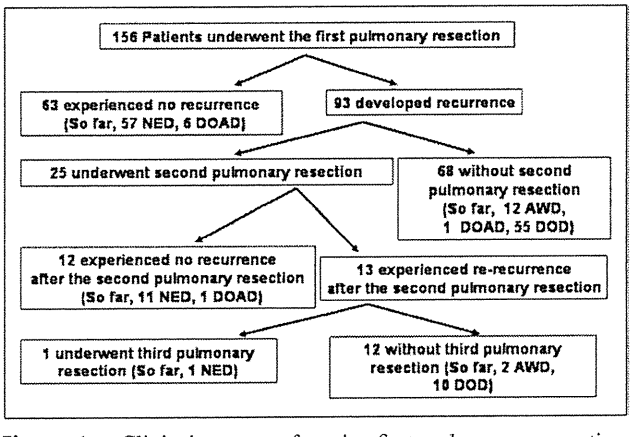

Figure 1 Clinical course after the first pulmonary resection. AWD, alive with disease; DOAD, died of another disease; DOD, died of disease; NED: no evidence of disease.

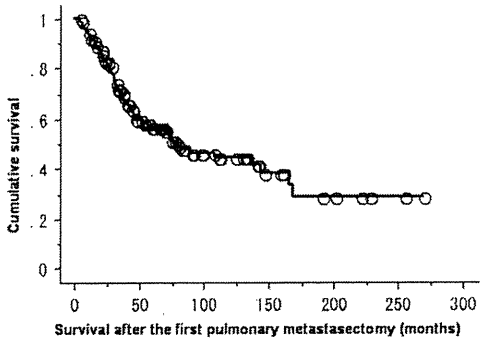

Figure 2 Overall survival of patients after the first pulmonary resection. The cumulative 3-year, 5-year, and 10-year survival rates after the first pulmonary resection were 71.4%, 56.2%, and 44.0%, respectively.

Analysis of prognostic factors for the first pulmonary resection

The following factors were selected for the univariate analysis of survival: sex, age, pathological stage of the primary CRC according to the Dukes classification, the location of the primary tumor, the histology of the primary tumor (well-differentiated adenocarcinoma/moderately differentiated adenocarcinoma or other/unknown), the interval between primary resection and first pulmonary resection (<24 or ≥24 months), the prethoracotomy serum carcinoembryonic antigen (CEA) level (<5 or ≥5 ng/mL), a history of surgical treatment for extrathoracic recurrence, repeat pulmonary resection (yes/no), the number of resected metastases, the site of metastasis (unilateral/bilateral), the maximum tumor size (≤3 or >30 mm), the hilar or mediastinal lymph node metastasis (yes/no), and the type of resection (sublobar resection/lobectomy/pneumonectomy). Table 2 shows the results of the univariate analysis. Significant relationships ($P < .05$) were found between survival and the following factors: histology of the primary tumor, prethoracotomy serum CEA levels, the maximum tumor size, the type of resection (sublobar resection, lobectomy, or pneumonectomy), and hilar or mediastinal lymph node metastasis. The significant factors identified by the univariate analysis were then subjected to a multivariate analysis (Table 3). A moderately differentiated carcinoma or other histological type, an elevated prethoracotomy serum CEA level (≥5 ng/mL), and hilar or mediastinal lymph node metastasis were found to be independent and significant determinants of a poor prognosis.

Table 3 Multivariate analysis of prognosis after the first pulmonary resection

Variable	*P* value	Risk ratio	95% confidence interval
Histology of primary tumor: moderately differentiated adenocarcinoma or others	.03	1.75	1.06–2.89
Prethoracotomy serum CEA level: ≥5 ng/mL	.01	1.87	1.16–3.03
Maximum tumor size: >30 mm	.1	1.53	.92–2.52
Hilar or mediastinal lymph node metastasis: yes	.04	2.12	1.05–4.29

Details of repeat pulmonary resections

Twenty-one patients who had pulmonary metastasis and 4 patients who had surgical margin relapse after the first pulmonary resection underwent repeat pulmonary resections. There was no operative major morbidity or

Table 2 Univariate analysis of the prognosis after the first pulmonary resection

Factors	n	5-year survival	*P* value
Sex (male/female)	91/65	49.7/65.2	NS
Age at first pulmonary resection (<63/≥63 y)	76/80	61.2/50.5	NS
Stage of primary tumor (Dukes ABC/D/unknown*)	124/21/11	55.3/49.6	NS
Location of primary tumor (colon/rectum)	74/82	56.6/55.5	NS
Histology of primary tumor (Well/moderately differentiated adenocarcinoma or others/unknown*)	65/88/3	61.9/51.6	.04
Interval between primary resection and first pulmonary resection (<24/≥24 months)	64/92	53.3/58.1	NS
Prethoracotomy serum CEA level (<5/≥5 ng/mL)	90/66	65.2/43.1	.006
History of surgical treatment of extrathoracic recurrence (yes/no)	41/115	46.3/59.2	NS
Repeat pulmonary resection (yes/no)	25/131	64.0/54.5	NS
Number of resected metastases (solitary/multiple)	100/56	60.0/49.9	NS
Site of metastasis (unilateral/bilateral)	130/26	61.3/33.5	NS
Maximum tumor size (≤30/>30 mm)	115/41	59.9/46.1	.01
Type of resection (sublobar resection/lobectomy or pneumonectomy)	99/57	57.7/53.7	NS
Hilar or mediastinal lymph node metastasis (yes/no)	15/141	28.1/59.4	.006

NS = not significant.
*Excluding unknown cases.

Table 4 Patient characteristics and details of repeat pulmonary resections

Characteristics	n
Sex (male/female)	
Male	17
Female	8
Age at second pulmonary resection (y)	
Mean	61
Range	47–79
Stage of primary tumor	
Dukes B	6
Dukes C	11
Dukes D	5
Unknown	3
Location of primary tumor	
Colon	8
Rectum	17
Histology of primary tumor	
Well-differentiated adenocarcinoma	11
Moderately differentiated adenocarcinoma	11
Others	2
Unknown	1
Interval between first and second pulmonary resection (months)	
Median	20
Range	5–57
Serum CEA level (ng/mL) before second pulmonary resection	
<5	18
≥5	7
Number of resected metastases at second pulmonary resection	
1	16
2	4
≥3	5
Site of metastasis	
Unilateral	23
Bilateral	2
Site of recurrence	
Pulmonary metastasis	21
Surgical margin relapse	4
Maximum tumor size (mm) at second pulmonary resection	
≤30	20
>30	5
Type of resection at second pulmonary resection	
Sublobar resection	16
Lobectomy or pneumonectomy	9
Hilar or mediastinal lymph node metastasis at second pulmonary resection	
Yes	23
No	2

mortality in the present patients. The surgical mode of second pulmonary resection was a wedge resection in 8 patients, segmentectomy in 8 patients, completion lobectomy in 4 patients, lobectomy in 3 patients, and completion pneumonectomy in 2 patients. All repeat pulmonary metastasectomies were curative resections. The median time interval between the first and repeat pulmonary resection was 20 months (range, 5–57 months). The mean patient age at the time of second pulmonary resection was 61 years (range, 47–79 years of age). Table 4 summarizes the patient characteristics and details of repeat pulmonary resections. The median time interval between second pulmonary resection and either death or the latest follow-up in the present series was 20 months (range, 1–238 months).

Overall survival of the patients after second pulmonary resection

The cumulative 3- and 5-year survival rates after the second pulmonary resection were 54.1% and 42.1%, respectively (Fig. 3).

Analysis of the prognostic factors for repeat pulmonary resection

The following factors were selected for a univariate analysis of survival for repeat pulmonary resection: sex, age at second pulmonary resection, pathological stage of the primary CRC according to the Dukes classification, location of primary tumor, histology of primary tumor, interval between first and second pulmonary resection (<20 or ≥20 months), serum CEA level before second pulmonary resection (<5 or ≥5 ng/mL), the number and site of resected metastases at the second pulmonary resection, site of recurrence (pulmonary metastasis/surgical margin relapse), maximum tumor size at second pulmonary resection (≤30 or >30 mm), type of resection at second pulmonary resection (sublobar resection, lobectomy, or pneumonectomy), and hilar or mediastinal lymph node metastasis at the time of the second pulmonary resection (Table 5). Significant relationships were identified only between hilar or mediastinal lymph node metastasis at the second pulmonary resection and survival after the repeat pulmonary resection.

Comments

In our hospital, pulmonary metastasectomies have been performed for various diseases including CRC, soft-tissue

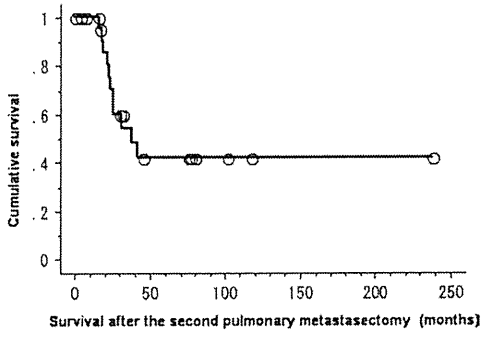

Figure 3 Overall survival rates of patients after the second pulmonary resection. The cumulative 3- and 5-year survival rates after the second pulmonary resection were 54.1% and 42.1%, respectively.

Table 5 Univariate analysis of the prognosis for repeat pulmonary resections

Factors	N	5-year survival	*P* value
Sex (male/female)	17/8	46.2/33.3	NS
Age at second pulmonary resection (<61 y/≥61 y)	13/12	45.7/40.0	NS
Stage of primary tumor (dukes BC/D/unknown*)	17/5/3	41.7/25.0	NS
Location of primary tumor (Colon/rectum)	8/17	42.9/41.0	NS
Histology of primary tumor (well/moderately differentiated adenocarcinoma or others/unknown*)	11/13/1	44.4/41.6	NS
Interval between first and second pulmonary resection (<20/≥20 months)	12/13	55.6/25.0	NS
Serum CEA level before second pulmonary resection (<5/≥5 ng/mL)	18/7	36.1/53.6	NS
Number of resected metastases at second pulmonary resection (solitary/multiple)	16/9	47.6/33.3	NS
Site of metastasis at second pulmonary resection (unilateral/bilateral)	23/2	44.3/0	NS
Site of recurrence (pulmonary metastasis/surgical margin relapse)	21/4	54.9/0	NS
Maximum tumor size at second pulmonary resection (≤30/>30 mm)	20/5	43.0/40.0	NS
Type of resection at second pulmonary resection (sublobar resection/lobectomy or pneumonectomy)	16/9	55.9/25.0	NS
Hilar or mediastinal lymph node metastasis at second pulmonary resection (yes/no)	2/23	0/46.8	.048

NS = not significant.
*Excluding unknown cases.

sarcoma, transitional cell carcinoma, and hepatocellular carcinoma according to the general eligibility criteria described in the Patients and Methods section and with good surgical outcomes.[18–23] The present study analyzed the outcomes in patients undergoing pulmonary metastasectomy from CRCs. The overall 5-year survival rate was 56.2%, which was consistent with the 24% to 71.2% range previously reported.[4–15]

Twenty-five patients who met the criteria described for this study underwent a second pulmonary metastasectomy and had a 5-year survival rate after the second resection of 42.1%. Table 6 compares the outcomes in studies involving more than 10 patients undergoing repeat pulmonary resection of metastatic CRCs.[5,9,11,13–15,17] The overall 5-year survival rates after the second pulmonary resection in these previous reports ranged from 23% to 52.1%. Considering the results of the current study, good surgical outcomes were achieved by repeat pulmonary resections of metastatic CRCs.

Many studies[5,8,9,14,24] showed no association between repeat pulmonary resection and poor survival by a multivariate analysis for survival after the first pulmonary resection. In addition, previous studies[5,13] of repeat pulmonary resection patients identified no increase in the risk of morbidity or mortality with a repeat pulmonary resection compared with the initial resection. In the present study, there was no major operative morbidity or mortality for patients undergoing repeat pulmonary resection even in 2 patients who had undergone completion pneumonectomies. These findings indicated that repeat pulmonary resection for metastatic CRC patients is a safe procedure that provides satisfactory patient outcomes.

In the present study, the poor prognostic factors for the first pulmonary resection of metastatic CRC included a moderately differentiated carcinoma or other histological type, an elevated prethoracotomy serum CEA level (≥5 ng/mL), and hilar or mediastinal lymph node metastasis according to a multivariate analysis. The prethoracotomy serum CEA levels were the most consistently reported prognostic factor for pulmonary metastasectomy from CRC.[4–6,9,12,19,24–28] We previously reported that the prethoracotomy CEA level was the most useful prognostic factor, and an elevated serum CEA level is associated with extrathoracic metastasis after pulmonary metastasectomy from CRCs. A number of reports[4,5,25,29] associated the presence of hilar or mediastinal lymph node metastases with a poor patient prognosis. Several authors[7,25] have shown results consistent with the present study, showing that a histological diagnosis for the primary CRC of well-differentiated adenocarcinoma is an independent significant prognostic factor after the first pulmonary resection of CRC.

Although prognostic factors for the first pulmonary resection of metastatic CRC have been well studied, the predictive factors for repeat pulmonary resection have not been sufficiently investigated. Ogata et al[13] reported that patients with extrathoracic recurrence before a second pulmonary metastasectomy or with mediastinal lymph node metastasis had a poorer prognosis. Welter et al[14] identified an increasing number of metastases as a poor prognostic factor for repeat pulmonary resection for metastatic CRC patients by a multivariate analysis, whereas Park et al[15] showed an association between elevated serum preoperative CEA levels and a poor prognosis by a univariate analysis.

Interestingly, the present study showed that the prognostic factors for the first and repeat pulmonary resection are different. Hilar or mediastinal lymph node involvement is consistently associated with a poor prognosis for both the first and repeat pulmonary resections, whereas the histological type of the primary CRC and the prethoracotomy serum CEA level does not significantly affect patient survival after second pulmonary resection. The differences in the prognostic factors for the first and repeat pulmonary resections depend on the differences between the characteristics of these 2 cohorts. The characteristics between these 2 cohorts

Table 6 Comparison of the surgical outcome of studies of patients undergoing repeat pulmonary resections for metastatic CRC

Author	Year	Total number of patients	Number of patients who underwent repeat pulmonary resection	#1	#2	Prognostic factor for second pulmonary resection	#3
Saito	2002	165	23	54.6	52.1	NA	NA
Pfannschmidt	2003	167	24	24.5*	NA	NA	NA
Vogelsang	2004	75	18	NA	NA	NA	NA
Ogata	2005	76	14	NA	23	Extrathoracic recurrence before second pulmonary metastasectomy, mediastinal lymph node metastasis is associated with poor prognosis	7/11
Welter	2007	169	33	53.8	37.1	Number of metastases by a multivariate analysis	NA
Lee	2007	59	13	NA	NA	NA	NA
Kim	2008	69	28	29	NA	NA	NA
Park	2009	202	48	79.3	NA	Elevated preoperative serum CEA level by univariate analysis	28/NA
Our series	2010	156	25	64	42.1	Lymph node involvement by a univariate analysis	10/13

NA = not available.
*Curative resection only.
#1: Five-year survival rate of patients who underwent repeat pulmonary resections after the first pulmonary resection.
#2: Five-year survival rate of patients who underwent repeat pulmonary resections after the second pulmonary resection.
#3: Total number of patients who experienced recurrence after second pulmonary resections.

reflect different patterns of recurrence after pulmonary resection. The proportion of pulmonary metastasis of patients who experienced recurrence after repeat resection was higher than after the first resection in the present study. Pulmonary metastasis after the second pulmonary resection was found in 10 patients (77%) out of 13 patients who experienced recurrence and in 39 of 93 patients (42%) who experienced recurrence after the first pulmonary resection in the present study. This higher proportion of pulmonary metastasis of patients who experienced recurrence after repeat resection is also observed in Ogata's series[13] in which the proportions was 64% (7/11) and 33% (25/75). It was speculated that patients who underwent repeat pulmonary resection are highly selected patients whose disease tended to metastasize to the lung without extrathoracic lesions after the first pulmonary resection.

Based on the data of the present study, it is speculated that repeat pulmonary resection for metastatic CRC patients with lymph node involvement should be avoided. Therefore, the preoperative assessment of lymph node involvement is important for repeat pulmonary resection. In our hospital, patients with apparent mediastinal lymph node metastases as determined by preoperative radiologic examinations were excluded as candidates for pulmonary metastasectomy. Before 2006, lymph node involvement was generally assessed by a CT scan. In 2006, FDG-PET/CT was introduced in our hospital for hilar and mediastinal lymph node staging in patients with metastatic lung tumors. One hundred thirty patients of 156 patients underwent a first pulmonary resection during this period in the present study. Fourteen of 130 patients (11%) in the CT era had lymph node involvement, whereas only 1 of 26 patients (4%) in the FDG-PET/CT era had lymph node involvement. Although the superior diagnostic performance of FDG-PET/CT compared with CT could not be shown because of the retrospective nature of the present study, we recommend the use of FDG-PET/CT for the preoperative assessment of patients with metastatic CRC.

The present study had several limitations. The analyses were performed on a large number of patients treated over several decades with changing chemotherapeutic regimens, and it is possible that reported outcomes were partly influenced by the medical treatments. Patients with recurrence after pulmonary resection who received more recently developed treatments, such as antiangiogenic therapy with bevacizumab combined with oxaliplatin-based chemotherapy,[3] may survive for a longer period than those who received an older regimen of chemotherapy regardless of the other factors that were analyzed. However, the significance and influence of chemotherapy on patient outcomes remains difficult to establish in the present study because of its retrospective nature.

Conclusions

Repeat pulmonary resection for metastatic CRC is a safe procedure that provides satisfactory outcomes. The prognostic factors for the first and repeat pulmonary resection are different. Prethoracotomy serum CEA levels and the histological type of primary CRC affect survival after the first pulmonary resection but do not affect survival after repeat pulmonary resection. Hilar or mediastinal lymph node involvement is consistently associated with a poor prognosis after the first and repeat pulmonary resections.

The preoperative assessment of lymph node involvement is important for both the first and repeat pulmonary resection. Aggressive repeat resection is justified for carefully selected patients.

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Lung Cancer 71 (2011) 333–337

Contents lists available at ScienceDirect

Lung Cancer

journal homepage: www.elsevier.com/locate/lungcan

Occult mediastinal lymph node metastasis in NSCLC patients diagnosed as clinical N0-1 by preoperative integrated FDG-PET/CT and CT: Risk factors, pattern, and histopathological study

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ARTICLE INFO

Article history:
Received 27 March 2010
Received in revised form 7 June 2010
Accepted 10 June 2010

Keywords:
Computed tomography
Lung cancer
Diagnosis
Positron emission tomography

ABSTRACT

Background: Integrated F18-fluorodeoxyglucose positron emission tomography/computed tomography (FDG-PET/CT) is widely used for mediastinal lymph node (MLN) staging in patients with non-small cell lung cancer (NSCLC). However, FDG-PET/CT has certain limitations. Prediction of occult MLN metastasis could allow selection of candidates for preoperative cervical mediastinoscopy or endobronchial ultrasound-guided transbronchial needle aspiration. This study defined risk factors for occult MLN metastasis in patients with NSCLC patients who were diagnosed as clinical N0-1 by preoperative integrated FDG-PET/CT and CT.

Methods: Consecutive patients with NSCLC who underwent staging using integrated FDG-PET/CT as an adjunct to CT prior to lung resection from October 2006 to September 2009 were evaluated retrospectively. The prevalence of MLN metastasis in patients diagnosed as clinical N0-1 was analyzed according to clinicopathological factors such as tumor location, tumor size, histology, and FDG uptake by the primary tumor. Risk factors for occult MLN metastasis were defined by multivariate analysis. Patterns of occult MLN metastasis were also analyzed and the involved MLNs were further examined histopathologically.

Results: The incidence of MLN metastasis was 11% (24 patients of 224). Multivariate analysis identified adenocarcinoma ($P = 0.04$), tumors located in upper or middle lobe ($P = 0.02$), tumor size >3 cm ($P = 0.01$), and SUV_{max} of primary tumor >4.0 g/ml ($P = 0.04$) as significant risk factors for MLN metastasis. The pattern of occult MLN metastasis was typical for NSCLC cases. The size of metastatic foci were small, with 68% of foci smaller than 4.0 mm.

Conclusions: The present study demonstrated that adenocarcinoma, tumors located in the upper or middle lobe, tumor size >3 cm, and SUV_{max} of primary tumor >4.0 g/ml are risk factors for occult MLN metastasis in patients with NSCLC who were diagnosed as clinical N0-1 by preoperative integrated FDG-PET/CT and CT. Patients with tumors located in the right upper or middle lobe are considered candidates for cervical mediastinoscopy because the involved metastatic mediastinal lymph nodes are easily accessible by these modalities.

1. Introduction

Lung cancer is the leading cause of cancer-related deaths in the Western world [1]. Despite advances in surgical management, chemoradiotherapy, and early diagnosis, the prognosis for patients with lung cancer remains poor. Accurate staging for non-small cell lung cancer (NSCLC) patients is important for both assessing prognosis and selecting the optimal therapy. Mediastinal lymph node (MLN) staging is particularly critical because survival is improved in patients with stage IIIA disease who undergo chemoradiotherapy followed by surgery, compared to those undergoing with surgery alone [2–4].

Chest computed tomography (CT) and cervical mediastinoscopy have been the gold-standard modalities for MLN staging in NSCLC patients. F18-fluorodeoxyglucose positron emission tomography (FDG-PET) has become increasingly utilized for MLN staging in

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doi:10.1016/j.lungcan.2010.06.008

NSCLC patients. To overcome the inherent disadvantages of FDG-PET such as poor quality of the anatomical information, new imaging systems using integrated FDG-PET/CT were developed recently [5]. This integrated approach provides higher sensitivity compared with CT alone in MLN staging for NSCLC.

A practical approach adopted in many centers is scheduling patients with negative mediastinal uptake by integrated FDG-PET/CT for resection [6]. However, the incidence of occult MLN metastasis in NSCLC patients showing negative uptake by FDG-PET/CT is 7–16% [6–8]. Several studies therefore investigated factors associated with occult MLN metastasis in NSCLC patients [6–8], since a successful prediction could select candidates for either preoperative cervical mediastinoscopy or endobronchial ultrasound-guided transbronchial needle aspiration. However, these studies were limited in that no histological examination of the involved MLNs was undertaken nor was a source of the negative result by integrated FDG-PET/CT discussed sufficiently.

The aim of the present study was to define risk factors for occult MLN metastasis in patients with NSCLC diagnosed as clinical N0-1 by preoperative integrated FDG-PET/CT and CT. Histopathological examination of the involved MLNs was conducted to examine why these lymph nodes were diagnosed as negative. The patterns of occult MLN metastasis were also analyzed.

2. Patients and methods

2.1. Patient eligibility and staging

The study retrospectively evaluated patients with NSCLC in our hospital who underwent staging by integrated FDG-PET/CT as an adjunct to CT from October 2006 to September 2009. No patient underwent preoperative mediastinoscopy in this period. The following patients were excluded from the present study: those who underwent limited resection (wide-wedge resection or segmentectomy; $n = 144$), patients with diabetes mellitus ($n = 39$), patients who received neo-adjuvant chemotherapy ($n = 11$), and patients with positive mediastinal uptake on integrated PET-CT or enlarged MLNs on CT (i.e., clinical N2/N3; $n = 39$). The remaining 224 patients were staged as clinical N0 or N1 by integrated FDG-PET/CT and CT, and underwent resection with systematic lymph node dissection. The preoperative CT, integrated FDG-PET/CT, and pathological findings were reviewed. Preoperative clinical staging and postoperative pathological staging was based on the 1997 update of the TNM staging system [9]. The study group comprised 122 men and 102 women age from 30 to 83 years (mean age of 65.5 years). Table 1 details the patient characteristics and preoperative tumor evaluations.

2.2. CT imaging

All studies (CT and integrated FDG-PET/CT) were interpreted independently. CT examination was performed using a helical scanner (LightSpeed VCT, General Electric, Waukesha, WI; Aquilion 16, Toshiba, Tokyo, Japan), and all patients had contrast-enhanced CT. Lymph nodes were interpreted as positive if >1 cm across the short-axis diameter. All positive nodes were localized according to the lymph node stations, based on the classification by Naruke et al. [10].

A tumor was deemed central if its center was located in the inner 1/3 of the lung parenchyma (adjacent to the mediastinum) on transverse CT imaging. Non-centrally located tumors were identified as those centered in the outer 2/3 of the lung parenchyma on transverse CT imaging. All CT images were performed within 4 weeks of surgery.

Table 1
Characteristics of patients and tumors ($n = 224$).

Characteristics	Distribution (%)
Sex	
Male	122 (54)
Female	102 (46)
Age (years)	
Mean ± SD	65.5 ± 8.9
Range	30–83
Smoking status	
Smoker	131 (58)
Never smoker	93 (42)
Concurrent lung disease[a]	
Present	40 (18)
Absent	184 (82)
Elevated serum CEA level (>5 ng/ml)	
Yes	68 (30)
No	156 (70)
Lobar distribution of the tumor	
RUL	100 (45)
RML	12 (5)
RLL	38 (17)
LUL	45 (20)
LLL	29 (13)
Location of the tumor	
Central	18 (8)
Non-central	206 (92)
Positive N1 node on PET/CT	
Yes	39 (17)
No	185 (83)
Tumor size (cm)	
Median	3.0
Mean ± SD	3.1 ± 1.5
Range	0.8–9.0
SUV_{max} of primary tumor	
Median	4.0
Mean ± SD	5.6 ± 5.1
Range	0–22.8

[a] Concurrent lung disease includes interstitial lung disease, chronic obstructive pulmonary disorder, bronchial asthma, and tuberculosis.

2.3. Integrated FDG-PET/CT imaging

Patients were asked to fast, except for glucose-free oral hydration, for at least 5 h before the injection of ^{18}F-FDG (3.5 MBq/kg body weight). After injection of the tracer, patients were kept lying comfortably on a bed. No urinary bladder catheterization was performed and no oral muscle relaxants were administered. Whole-body PET/CT fusion scanning was performed 1 h after the injection, using a PET/CT system (Discovery LS, General Electric, Waukesha, WI; Biograph Duo LSD, Siemens-Asahi Medical Technologies, Tokyo, Japan). PET, CT, and integrated PET/CT images were available for review, displayed in axial, coronal, and sagittal planes. The FDG uptake of tumor was visually compared with that of the surrounding tissue in areas devoid of prominent artifacts and overlapping increased FDG uptake organs. A team of experienced radiologists reviewed the integrated FDG-PET/CT images independently from the CT data. Nodal uptake with a standardized uptake value (SUV_{max}) >2.5 were interpreted as positive. All integrated FDG-PET/CT imaging was performed within 4 weeks of surgery.

2.4. Surgical resection

All of the surgical resections and mediastinal nodal dissections were conducted by thoracic surgeons at Osaka Medical Center for Cancer and Cardiovascular Diseases. Systematic lymph node dissection was carried out.

Table 2
Postoperative pathological evaluation of the tumors.

Type of operation	
Lobectomy	213 (95%)
Bilobectomy	10 (4%)
Pneumonectomy	1 (1%)
Histopathological type	
Adenocarcinoma	180 (80%)
Squamous cell carcinoma	37 (17%)
Other types[a]	7 (3%)
Lymph node metastasis	
pN0	168 (75%)
pN1	32 (14%)
pN2	24 (11%)
Pathological stage after surgery[b]	
IA	101 (46%)
IB	64 (29%)
IIA	10 (4%)
IIB	21 (9%)
IIIA	23 (10%)
IIIB	5 (2%)

[a] Other histopathological types of NSCLC included adeno-squamous carcinoma, large cell carcinoma.
[b] Stage of disease was defined according to the 1997 update of TNM criteria established by UICC.

Table 3
Pattern of mediastinal lymph node involvement.

Case	Lobar distribution	pN2 station	Pathological N1 node
1	RUL	1,3	Negative
2	RUL	2	Negative
3	RUL	3	Positive
4	RUL	3	Negative
5	RUL	3,4	Positive
6	RUL	3,4	Positive
7	RUL	3,4	Negative
8	RUL	4	Negative
9	RUL	1,2,3,4	Positive
10	RUL	1,2,4,7	Positive
11	RUL	1,3,4,7	Positive
12	RUL	1,3,4,7	Positive
13	RML	3,7	Negative
14	RLL	7	Negative
15	LUL	4	Positive
16	LUL	4,5	Negative
17	LUL	5	Positive
18	LUL	5	Positive
19	LUL	5	Positive
20	LUL	5	Negative
21	LUL	5	Negative
22	LUL	5,6	Positive
23	LUL	5,6	Positive
24	LLL	7	Positive

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

2.5. Pathological examination and the size of metastases

All resected tumor specimens were examined by experienced pulmonary pathologists. Histological classification of NSCLC was based on the WHO classification [11]. The dissected lymph nodes were examined histologically following hematoxylin and eosin staining, and the long-axis diameters of the metastatic foci in all involved lymph nodes were measured. The lymph node with the largest metastatic foci was selected as a representative foci of each station containing metastases.

2.6. Statistical analysis

Statistical analysis was performed with Dr.SPSS II software (SPSS Japan, Tokyo, Japan). Univariate data analysis was conducted using Fischer's exact test or Pearson's chi-square test. Multivariate analysis was conducted using the logistic regression (backwards stepwise) method. *P*-values were considered statistically significant if <0.05.

3. Results

3.1. The incidence and pattern of MLN metastasis

The incidence of MLN metastasis in this study was 11% (24 of 224 patients). Table 2 details the postoperative pathological evaluation of the tumors. Of 24 patients with mediastinal node metastasis, multistation MLN metastasis was found in 12 patients (50%), while the other 12 patients showed on a single station (50%). Skip metastasis was found in 10 patients (42%). Forty-four MLN stations in total were involved. Table 3 indicated the patterns of MLN involvement. In patients whose tumors were located in the right upper lobe ($n=12$), 9 had metastasis in the superior lymph nodes (#1, #2, #3, #4) and 3 patients had metastases in both the superior and inferior (#7) lymph nodes. One patient whose tumor was located in the right middle lobe had metastasis in #3 and #7 lymph nodes, while a patient with a tumor in the right lower lobe had metastasis in #7 lymph node. In patients whose tumors were located in left upper lobes ($n=9$), 7 patients had metastasis in the aortic lymph nodes (#5, #6), 1 patient had metastasis in #4 lymph nodes, and 1 patient had metastasis in #4 and #5 lymph nodes. Finally, a patient whose tumor was located in the left lower lobe had metastasis in #7 lymph node.

3.2. Analysis of risk factors associated with MLN metastasis

Table 4 summarizes the results of univariate analysis for factors associated with MLN metastasis. Factors that are significantly associated with MLN metastasis are: never smoked ($P=0.03$), tumor located in the upper or middle lobe ($P=0.01$), tumor >3 cm ($P=0.005$), SUV_{max} of primary tumor >4.0 g/ml ($P=0.0498$), and adenocarcinoma ($P=0.04$). The multivariate risk-factor analysis (Table 5) identified adenocarcinoma ($P=0.04$), tumors located in the upper or middle lobe ($P=0.02$), tumor >3 cm ($P=0.01$), and SUV_{max} of primary tumor >4.0 g/ml ($P=0.04$) as risk factors for MLN metastasis.

3.3. A Pathological examination and the size of metastases

The size of metastatic foci across 44 stations ranged from 0.5 to 9 mm with a mean value of 3.7 ± 2.0 mm (±SD). Thirty of the 44 involved stations (68%) had metastatic foci smaller than 4.0 mm. Table 6 details the size distribution of the metastatic foci.

4. Discussion

The present study defined risk factors for occult metastasis in patients with NSCLC diagnosed as clinical N0-1 by preoperative integrated FDG-PET/CT and CT. The patterns of occult MLN metastasis were also analyzed, and the involved MLNs were examined histologically. The incidence of MLN metastasis in our series of patients was 11%. This finding was concordant with comparable previous studies; Al-Sarraf et al. [6] reported the incidence of occult N2 disease in similar patients as 16%, while Cerfolio et al. [12] reported a 14% incidence, although when they limited the analysis to clinical stage I patients, the incidence decreased to 7% [7]. Risk factors for occult N2 disease reported in these previous studies were as follows: adenocarcinoma [7], tumors located in right upper lobe [6], larger tumor size [7], a high SUV_{max} of the primary tumor [7,12], centrally located tumors [6,7], positive N1 nodes on PET [6],

Table 4
Univariate analysis for factors associated with mediastinal lymph node metastasis.

Variable	Pathological N2 (n=24)	Pathological N0-1 (n=200)	P-value
Sex			
Male	9	113	
Female	15	87	N.S.
Age (years)			
≥65	13	114	
<65	11	86	N.S.
Smoking status			
Smoker	9	122	
Never smoker	15	78	0.03
Concurrent lung disease			
Present	2	38	
Absent	22	162	N.S.
Elevated serum CEA level (>5 ng/ml)			
Yes	7	61	
No	17	139	N.S.
Lobar distribution of the tumor[a]			
Upper or middle lobe	22	135	
Lower lobe	2	65	0.01
Location of the tumor			
Central	2	16	
Non-central	22	184	N.S.
Positive N1 node on PET/CT			
Yes	3	36	
No	21	164	N.S.
Tumor size (cm)			
>3.0	18	90	
≤3.0	6	110	0.005
SUV_{max} of primary tumor			
>4.0	16	91	
≤4.0	8	109	0.0498
Histopathological type			
Adenocarcinoma	23	157	
Non-adenocarcinoma	1	43	0.04

[a] Upper or middle lobe includes right upper lobe, right middle lobe, and left upper lobe. Lower lobe includes right lower lobe, left lower lobe.

Table 5
Multivariate analysis for risk factors for mediastinal lymph node metastasis.

Variable	Odds ratio	Confidence interval	P-value
Adenocarcinoma	9.26	1.13–76.9	0.04
Located in upper or middle lobe	6.29	1.36–29.4	0.02
Tumor size >3 cm	4.18	1.48–11.76	0.01
SUV_{max} of primary tumor >4.0	2.79	1.04–7.52	0.04

and poorly differentiated histology [12]. A limitation of these studies was the lack of histological examination of the involved MLNs and insufficient consideration of why the involved lymph nodes showed as negative by integrated FDG-PET/CT.

Adenocarcinoma was also identified as a risk factor for occult MLN metastasis in the present patient cohort. Interestingly, Lee et al. [7] reported similar data in that all of their 16 patients with pathological N2 disease showed adenocarcinoma as the primary tumor cell type. On the contrary, in the Al-Sarraf and co-workers' series [6], the primary tumor cell type did not affect the incidence of occult MLN metastasis. A major difference between their series and ours is the inclusion of patients with enlarged lymph nodes (>1 cm) on CT in our analysis. In the comparative study [6], 8 of 25 patients with pathological N2 disease in their series had enlarged lymph nodes (>1 cm) on CT, and 12 of the 25 had non-adenocarcinoma tumors. It is reported that lymph node metastases from adenocarcinoma were of normal size (1 cm ≤ across the short-axis diameter) more frequently than those from squamous cell carcinoma [13,14]. Therefore, we speculate that patients with MLN metastasis from squamous cell carcinoma tended to be excluded in our series and included in the series of patients reported on by Al-Sarraf and co-workers [6].

Tumor location in the upper or middle lobe also proved to be a risk factor for occult MLN metastasis in the present study. A predisposition of lobar distribution was also observed in the previous study by Al-Sarraf and co-workers, with the right upper lobe dominating in their series. The other risk factors for occult MLN metastasis were identified as tumor size >3 cm, SUV_{max} of primary tumor >4.0 g/ml. It is well known that the incidence of MLN metastasis increased as the tumor size increased [15]. The relationship between SUV_{max} of the primary tumor and incidence of lymph node metastasis has also been investigated previously. Downey and colleagues [16] reported higher SUV_{max} of the primary tumors in patients with pathological nodal involvement than in patients without nodal involvement, while Cerfolio and colleagues [8] showed that SUV_{max} of lung tumors increased as the cancer progressed from N0 to N3.

In contrast to the report of Al-Sarraf et al. [6], positive N1 nodes on PET was not a risk factor for MLN metastasis in the present study. This difference might be attributable to the relatively high incidence of skip metastasis in the present series (42%).

To overcome a limitation of similar previous studies, we conducted histological examination of the involved MLNs. This additional analysis demonstrated that metastatic foci of occult MLN metastases were small; of 44 stations, the size of foci ranged from 0.5 to 9 mm with a mean value of 3.7 ± 2.0 mm, and 30 of the 44 involved stations (68%) contained foci of <4.0 mm diameter. This finding is supported by previous studies wherein the metastatic foci of false-negative lymph nodes by PET were small. Takamochi et al. [17] reported such foci to be 1–7.5 mm (mean, 3.4 mm; n=12), while Nomori et al. [18] found that false-negative (n=8) and true-positive (n=28) lymph nodes on PET contained foci ranging from 0.5 to 9 mm (mean, 3 mm) and 4–18 mm (mean, 10 mm), respectively. No metastatic foci smaller than 4 mm were detected with PET.

The pattern of occult MLN metastasis shown in the present study was identical to the typical distribution in NSCLC patients reported by Naruke et al. [19] These authors reviewed 1815 patients who underwent systematic lymph node dissection, and examined which nodes had the highest likelihood of metastasis. Distribution of MLN metastasis from each lobe in their series was as follows: right upper lobe tumor, #3 (12.3%) and/or #4 (8%); right middle lobe tumor, #3 and/or #7 (16.4%); right lower lobe tumor, #7 (13.7%); left upper lobe tumor, #5 (12.3%) and/or #6 (6.7%); and left lower lobe tumor, #7 (11.9%). Based on our data, patients with tumors in the right upper or middle lobe are potential candidates for cervical mediastinoscopy because their possible metastatic mediastinal lymph nodes (#3, #4, #1, #2, #7) are easily accessible by cervical mediastinoscopy or endobronchial ultrasound-guided transbronchial needle aspiration. Skip metastasis was found in 10 patients (42%) in

Table 6
Distribution of size of metastatic foci.

	Size (mm)											
	<1.0	<2.0	<3.0	<4.0	<5.0	<6.0	<7.0	<8.0	<9.0	<10.0	≥10.0	Total
No.	1	5	12	12	5	3	2	1	0	3	0	44

the present series, which is a relatively high incidence compared to other reports for NSCLC patients (25–29%) [15]. In these 10 patients, it is speculated that metastatic MLNs were negative for integrated FDG-PET/CT and CT because they are the first nodal target of lymph from a primary tumor site and metastatic foci were still small.

Because of the small size of the metastatic foci, occult MLNs are considered difficult to be detected by endobronchial ultrasound-guided transbronchial needle aspiration. Therefore, we recommend cervical mediastinoscopy for the patients with tumors in the right upper or middle lobe who have risk factors for occult MLN metastasis.

5. Conclusions

The present study demonstrated that adenocarcinoma, tumors located in the upper or middle lobe, tumor size >3 cm, and SUV_{max} of primary tumor >4.0 g/ml are risk factors for occult MLN metastasis in patients with NSCLC diagnosed as clinical N0-1 by preoperative integrated FDG-PET/CT and CT. The metastatic foci of involved stations were small, with 68% of foci measuring <4.0 mm. The pattern of occult MLN metastasis discerned in the present study was typical for reported distribution of metastatic foci in NSCLC patients. Patients with tumors in the right upper or middle lobe are considered candidates for cervical mediastinoscopy because their metastatic mediastinal lymph nodes (#3, #4, #1, #2, #7) are easily accessible by these modalities.

Conflict of interest statement

None of the authors has any financial or other potential conflict of interest.

Acknowledgement

This study was partly supported by a grant from Osaka Anticancer Foundation.

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Lung Cancer 73 (2011) 217–221

Contents lists available at ScienceDirect

Lung Cancer

journal homepage: www.elsevier.com/locate/lungcan

Alveolar space filling ratio as a favorable prognostic factor in small peripheral squamous cell carcinoma of the lung

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ARTICLE INFO

Article history:
Received 11 June 2010
Received in revised form
21 September 2010
Accepted 3 December 2010

Keywords:
Lung
Squamous cell carcinoma
Peripheral type
Alveolar space filling ratio
Prognostic factor
Microinvasive carcinoma

ABSTRACT

Introduction: Squamous cell carcinomas (SqCCs) of the lung can be divided into two types according to the location of primary site; one is central type and another is peripheral type. Many reports on the central type revealed the clinicopathological characteristics relating carcinogenesis, therapeutics and prognosis. On the other hand, those on the peripheral type are very a few and prognostic indicators of peripheral type have not been enough elucidated. The aim of this study was to clarify clinicopathological prognostic factors of small peripheral SqCCs of the lung 30 mm or less.
Materials and methods: We evaluated various 15 clinicopathological parameters in 81 patients with peripheral type SqCCs, which are defined as tumors located in or more peripheral from the third branching bronchus, measuring 30 mm or less in diameter.
Results: Univariate analyses were performed using the log lank test and multivariate analyses using logistic regression model. As a result, two factors had a statistically significant influence on outcome of the patients in the univariate analysis; no relapse was observed in the patients with the ratio of alveolar space filling (ASF) area to tumor area of 70% or more and the maximum diameter of invasive area measuring 10 mm or less in size ($P=0.0214$, $P=0.0373$, respectively). Meanwhile, multivariate analysis showed that the ASF ratio of 70% or more significantly affected the outcome of the patients ($P=0.0337$), however the maximum diameter of invasive area did not ($P=0.2136$). We could not show the unfavorable prognostic factor contributory to tumor relapse.
Conclusions: We have shown that the ASF ratio is a significantly favorable prognostic factor for small peripheral type. Especially the focally invasive tumors with ASF ratio of 70% or more might be classified as "a microinvasive carcinoma" of the peripheral SqCCs of the lung and tumors with ASF ratio 100% as noninvasive carcinoma.

1. Introduction

Lung cancer is the leading cause of cancer death in the United States and other countries, including Japan. Squamous cell carcinoma (SqCC) occupies approximately 30% in all lung cancers [1]. About 70% of SqCC has been reported to arise from central portion of the lung, whereas the remaining 30% does from periphery [2]. According to a statistical report by Kodama in Japan, the peripheral type of SqCC goes on increasing around 50% of all SqCCs of the lung [3]. Many reports of the central type SqCC have revealed the pathological and clinical characteristics relating therapeutics and prognosis, but those of the peripheral type have been very a few because the biological behavior has not been well evaluated [4–6].

Central type SqCC of the lung is generally believed to arise from bronchial dysplastic epithelium through the multiple steps of dysplasia–carcinoma sequence, which is the same well-known carcinogenic pathway as observed in uterine cervical neoplasia [7]. During this pathway, carcinoma in situ (CIS) is the earliest stage of the progression of central type SqCC and showed favorable prognosis. If the CIS lesion is localized, a non-surgical procedure like photodynamic therapy may be selected instead of operation. On the other hand, the carcinogenic pathway observed as dysplasia–CIS sequence in the central type has not been fully established in the peripheral SqCC of the lung, so that the standard treatment of peripheral SqCC in the early stage is confined to lobectomy at the present time.

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doi:10.1016/j.lungcan.2010.12.001

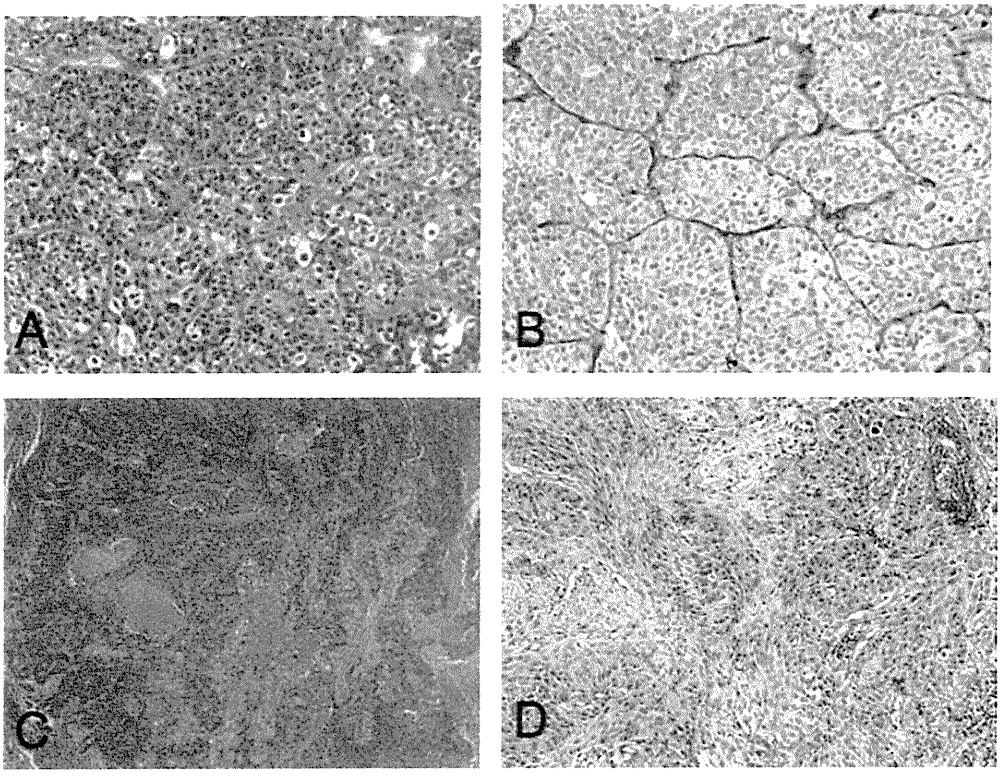

Fig. 1. Peripheral squamous cell carcinoma (SqCC) consisting of two types of area; alveolar space filling (ASF) area and non-ASF area. (A) Histology of alveolar space filling (ASF) area. Hematoxylin and eosin (H&E). (B) Elastic fiber framework of ASF area. Elastica von Gieson (EVG). (C) Histology of non-ASF area. H&E. (D) Destruction of elastic fiber framework was observed. EVG.

Recently, several studies of peripheral SqCC have been presented. Funai et al. studied 109 cases of peripheral SqCC and indicated that those can be divided into three subtypes based on two distinctive structural patterns based on the tumor growth pattern and the condition of the elastic fiber framework (EFF): the alveolar space filling (ASF) type, the expanding type and the mixed type consisting of the mixture of the former two types [5]. Pure ASF type tumors, which showed no destruction of elastic fiber framework, had a favorable prognosis, showed 100% 5-year survival and thought to be classified as CIS of peripheral SqCCs. Meanwhile, Maeshima et al. studied 101 cases of peripheral SqCCs and proposed three types based on the minimal tumor nest (MTN) pattern: large nest consisting of more than 6 tumor cells, small nest consisting of 2–5 tumor cells, or single cell [6]. They considered tumors with single cell invasive component appeared to be highly malignant, whereas those composed only of large tumor nest or small tumor nest components seemed to have a relatively low potential for malignancy and recurrence, despite these two types also being invasive.

We consider that the clarification of clinicopathological prognostic factors in the peripheral SqCCs leads to the prediction of recurrence or survival and contribution to increase of therapeutic selections. The aim of this study was to identify favorable and unfavorable clinicopathological factors for small peripheral SqCCs of the lung.

2. Materials and methods

2.1. Patients

Between January 1993 and September 2008, surgical resection was performed in 364 cases of lung SqCCs at Kanagawa Cancer Center Hospital. The subjects of the study were consecutive 81 patients with small peripheral SqCC of the lung measuring 30 mm or less in diameter. "Peripheral type" in this study was defined as tumors located in or more peripheral to the third branching bronchus.

The patients included 72 men and 9 women with a median age of 70.5 years (range, 47–84 years). We obtained the various clinical parameters from a medial record of each patient, including age, gender, smoking habit, exposure to asbestos, and limited or standard resection. The cases were classified according to the 7th edition of the Union International Against Cancer Staging System [8].

2.2. Pathologic review

All tumor slides stained with hematoxylin and eosin (H&E) and by the Elastica von Gieson method (EvG) for elastic fibers were reviewed by two of the authors (YW and TY) to confirm the histological differentiation, tumor stage, vessel invasion, lymphatic permeation, pleural involvement, presence or absence of usual interstitial pneumonia (UIP), the degree of lymphocytic inflammatory cell infiltration into the tumor nests, the size of necrotic area, the maximum diameter of invasive growth area which was characterized by the destruction of EFF and stromal cell reaction (Fig. 1), the proportion of ASF area to the entire tumor area (ASF ratio), and the minimal tumor nest (MTN) pattern. The degree of inflammation was defined as follows; mild lymphocytic tumor nest infiltration, the number of less than 10% of tumor cells per nest; moderate, 10 to less than 50%; severe, 50% or more. Definition of UIP met histological features of UIP pattern in the American Thoracic Society/European Respiratory Society international multidisciplinary consensus classification of idiopathic interstitial pneumonias [9]. ASF area showed tumor cells fill the alveoli and grow from one alveolus to another with no destruction of the elastic framework as if tumor cells penetrate the pores of Cohn and no desmoplastic