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4. Drilon A, Wang L, Hasanovic A, Suehara Y, Lipson D, Stephens P, et al. Response to Cabozantinib in Patients with RET Fusion-Positive Lung Adenocarcinomas. Cancer Discov 2013;3:630–5.
5. Takeuchi K, Soda M, Togashi Y, Suzuki R, Sakata S, Hatano S, et al. RET, ROS1 and ALK fusions in lung cancer. Nat Med 2012;18:378–81.
6. Mano H. ALKoma: a cancer subtype with a shared target. Cancer Discov 2012;2:495–502.
7. Lipson D, Capelletti M, Yelensky R, Otto G, Parker A, Jarosz M, et al. Identification of new ALK and RET gene fusions from colorectal and lung cancer biopsies. Nat Med 2012;18:382–4.
8. Kohno T, Ichikawa H, Totoki Y, Yasuda K, Hiramoto M, Nammo T, et al. KIF5B-RET fusions in lung adenocarcinoma. Nat Med 2012; 18:375–7.
9. Ju YS, Lee WC, Shin JY, Lee S, Bleazard T, Won JK, et al. A transforming KIF5B and RET gene fusion in lung adenocarcinoma revealed from whole-genome and transcriptome sequencing. Genome Res 2012;22:436–45.
10. Oxnard GR, Binder A, Janne PA. New targetable oncogenes in non-small-cell lung cancer. J Clin Oncol 2013;31:1097–104.
11. Kohno T, Tsuta K, Tsuchihara K, Nakaoku T, Yoh K, Goto K. RET fusion gene: Translation to personalized lung cancer therapy. Cancer Sci 2013;104:1396–400.
12. Travis WD, Brambilla E, Riely GJ. New pathologic classification of lung cancer: relevance for clinical practice and clinical trials. J Clin Oncol 2013;31:992–1001.
13. Travis WD, Brambilla E, Noguchi M, Nicholson AG, Geisinger KR, Yatabe Y, et al. International association for the study of lung cancer/ american thoracic society/european respiratory society international multidisciplinary classification of lung adenocarcinoma. J Thorac Oncol 2011;6:244–85.
14. Tsuta K, Kawago M, Inoue E, Yoshida A, Takahashi F, Sakurai H, et al. The utility of the proposed IASLC/ATS/ERS lung adenocarcinoma subtypes for disease prognosis and correlation of driver gene alterations. Lung Cancer 2013;81:371–6.
15. Warth A, Muley T, Meister M, Stenzinger A, Thomas M, Schirmacher P, et al. The novel histologic International Association for the Study of Lung Cancer/American Thoracic Society/European Respiratory Society classification system of lung adenocarcinoma is a stage-independent predictor of survival. J Clin Oncol 2012;30:1438–46.
16. Yoshizawa A, Motoi N, Riely GJ, Sima CS, Gerald WL, Kris MG, et al. Impact of proposed IASLC/ATS/ERS classification of lung adenocarcinoma: prognostic subgroups and implications for further revision of staging based on analysis of 514 stage I cases. Mod Pathol 2011;24:653–64.
17. Travis WD, Brambilla E, Muller-Hermelink HK, Harris CC, editors. World Health Organization classification of tumors: Pathology and genetics, tumours of lung, pleura, thymus and heart. Lyon, France: IARC Press; 2004.
18. Kim D, Salzberg SL. TopHat-Fusion: an algorithm for discovery of novel fusion transcripts. Genome Biol 2011;12:R72.
19. Falls DL. Neuregulins: functions, forms, and signaling strategies. Exp Cell Res 2003;284:14–30.
20. Fleck D, van Bebber F, Colombo A, Galante C, Schwenk BM, Rabe L, et al. Dual cleavage of neuregulin 1 type III by BACE1 and ADAM17 liberates its EGF-like domain and allows paracrine signaling. J Neurosci 2013;33:7856–69.
21. Dislich B, Lichtenthaler SF. The membrane-bound aspartyl protease BACE1: molecular and functional properties in Alzheimer's disease and beyond. Front Physiol 2012;3:8.
22. Majem M, Pallares C. An update on molecularly targeted therapies in second- and third-line treatment in non-small cell lung cancer: focus on EGFR inhibitors and anti-angiogenic agents. Clin Transl Oncol 2013;15:343–57.
23. Perez EA, Spano JP. Current and emerging targeted therapies for metastatic breast cancer. Cancer 2012;118:3014–25.
24. Nelson V, Ziehr J, Agulnik M, Johnson M. Afatinib: emerging next-generation tyrosine kinase inhibitor for NSCLC. Onco Targets Ther 2013;6:135–43.
25. Palanisamy N, Ateeq B, Kalyana-Sundaram S, Pflueger D, Ramnarayanan K, Shankar S, et al. Rearrangements of the RAF kinase pathway in prostate cancer, gastric cancer and melanoma. Nat Med 2010; 16:793–8.
26. Wilhelm SM, Adnane L, Newell P, Villanueva A, Llovet JM, Lynch M. Preclinical overview of sorafenib, a multikinase inhibitor that targets both Raf and VEGF and PDGF receptor tyrosine kinase signaling. Mol Cancer Ther 2008;7:3129–40.
27. Kettle R, Simmons J, Schindler F, Jones P, Dicker T, Dubois G, et al. Regulation of neuregulin 1beta1-induced MUC5AC and MUC5B expression in human airway epithelium. Am J Respir Cell Mol Biol 2010;42:472–81.

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Clinical Cancer Research

Druggable Oncogene Fusions in Invasive Mucinous Lung Adenocarcinoma

Takashi Nakaoku, Koji Tsuta, Hitoshi Ichikawa, et al.

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Pattern of Metastasis Outside Tumor-Bearing Segments in Primary Lung Cancer: Rationale for Segmentectomy

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Background. Patterns of intrapulmonary metastasis, particularly metastasis outside tumor-bearing segments, were investigated in lung cancer patients to address the rationale for segmentectomy.

Methods. In a consecutive series of patients who underwent resection of two or more pulmonary segments for primary lung cancer, intrapulmonary spread patterns, such as segmental/intersegmental node metastasis and pulmonary parenchymal metastasis, were pathologically examined.

Results. Eligible 244 lesions included 167 adenocarcinomas, 66 squamous cell carcinomas, and 11 large cell carcinomas. Pathologic stages included 0 to IA (n = 111), IB (n = 56), IIA (n = 31), IIB (n = 20), IIIA (n = 23), and IIIB to IV (n = 3); and N1 (n = 26) and N2 (n = 22). Intrapulmonary spread was observed in 24 cases (9.8%). Of these, metastasis outside tumor-bearing segments was only observed in 4 cases (1.6%), and such cancer spread was more frequently seen in cases with extrapulmonary (hilar to mediastinal) nodal metastasis (7.9%) than in cases without extrapulmonary metastasis (0.5%; $p = 0.01$). Metastasis outside tumor-bearing segments was not observed in 64 tumors with pure or mixed ground glass opacity features on computed tomography. Although tumor location (peripheral or central/intermediate) was not related to the incidence of metastasis outside tumor-bearing segments, intrapulmonary spread was observed in only 1 of 52 peripheral small (≤20 mm) tumors.

Conclusions. Metastasis outside tumor-bearing segments is rarely observed in cases with tumors (1) without extrapulmonary nodal metastasis and (2) with ground glass opacity or peripheral small (≤20 mm) features.

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Lobectomy with systematic nodal dissection has been a standard surgery for non-small cell lung cancer (NSCLC) for more than 50 years [1], and sublobar resection such as wedge resection or segmentectomy [2, 3] is an alternative for compromised patients as it was proved that sublobar resection is inferior to lobectomy in terms of local control as well as prognosis by the historical randomized controlled study conducted by the Lung Cancer Study Group in 1980s [4]. However, a great interest in limited resections has kept growing since small-sized and peripheral lung cancer has increased over time. A number of studies performed in the 1990s and 2000s demonstrated that segmentectomy is more radical than wedge resection [5–8], and segmentectomy would be as curative as lobectomy if peripheral and small-sized tumors were selected [9]. Now limited resections for such a type of NSCLC is one of the most important concerns of thoracic surgeons.

In 1889, William Ewart first described the units of bronchopulmonary segments, and Churchill and colleagues [10] stated that "the bronchopulmonary segment may replace the lobe as the surgical unit of the lung," based on their experience of lingular segment resection in 1939. Foster-Caster and Hoyle [11] reevaluated this theory based on radiologic findings, and defined the bronchopulmonary segment as that area of the lung supplied by a principal branch of a lobar bronchus. Nevertheless, the lymphatic spread hypothesis remains based on the traditional nodal cascade spread theory [12, 13]. For instance, lymphatic spread patterns should be primarily prograde; this theory provides the rationale for segmentectomy as a treatment for primary lung cancer. However, recently it has also become accepted that no definitive

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intrathoracic lymphatic spread pattern exists [14]; thus, whether segmentectomy is a feasible treatment for lung cancer considering intrapulmonary lymphatic spread patterns remains to be clarified. Furthermore, parenchymal metastasis must also be considered in addition to lymph node metastasis as a cause of lymphatic spread [15].

The aim of this study was to better understand the biology of intrapulmonary and regional lymph node metastasis of NSCLC by examination of intrapulmonary spread patterns, including nodal and parenchymal metastasis, in resected specimens.

Material and Methods

Patients

The Ethics Committee of Chiba University Graduate School of Medicine approved this research (no. 1589). From September 2009 to August 2012, a consecutive series of 346 patients with 406 lesions undergoing pulmonary resection for treatment of lung cancer was evaluated, and clinical and pathologic data were collected in a prospective setting. The TNM staging and lymph node station numbers were determined according to the seventh edition of the TNM classification for lung cancer (Union for International Cancer Control-7) [16]. The exclusion criteria of this study were resection of single segment or less, no systematic sampling or dissection during the surgery, ipsilateral multiple lesions, small cell lung cancer and rare histologic type of NSCLC, and preoperative cancer treatment including induction chemotherapy or chemoradiotherapy.

In all patients, radiologic findings and locations of tumors were defined by thin-section computed tomography (CT), which involved multidimensional slicing and reconstruction into axial, coronal, and sagittal views. Tumor location was also classified into three loci based on three-dimensional imaging: peripheral type was defined as the center of tumor being located in the outer third layer of the whole lung; central type was defined as the center of tumor being located in the inner third layer; and intermediate type was defined as the center of tumor being located between peripheral type and central type. Tumor CT findings were read by two or more radiologists and assigned to one of the following three groups based on axial CT imaging in a preoperative conference that all general thoracic surgeons and radiologists attend: pure ground grass opacity (GGO) type as 100% GGO appearance; solid type as 100% solid appearance without any GGO components; and mixed GGO type as any other patterns (any combination of solid and GGO components). Clinical information was collected from medical charts.

Pathologic Examination of Lymph Nodes

After identification of each bronchus and intersegmental veins that are segmental borders in the resected specimens, intrapulmonary nodes, including intersegmental nodes (station 13) and segmental nodes (station 14), were dissected by a well-trained thoracic surgeon immediately after surgery. Station 13 and station 14 nodes were identified and separately recorded as being inside or outside the tumor-bearing segment, and were subjected to pathologic examination (Fig 1). We also separately recorded extrapulmonary nodes, including hilar nodes (hilar, station 10; interlobar, station 11; and lobar, station 12) and mediastinal nodes (stations 1–9). We defined "skip N2 metastasis" as a mediastinal nodal metastasis without any hilar, lobar, or intrapulmonary node involvement. Intersegmental borders were determined by detection of intersegmental veins. The lung parenchyma was inspected after formalin fixation by pathologists, the resected lung was cut into 1-cm slices in the axial plane, and the pathologic parenchymal metastasis (pm) status was also diagnosed and recorded.

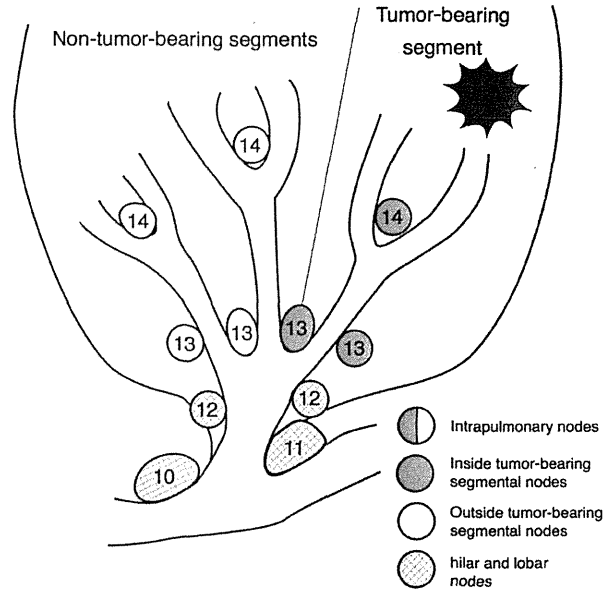

Fig 1. Schema of hilar and lobar, and intrapulmonary nodes. Intrapulmonary nodes (stations 13 and 14) were divided into two groups: inside tumor-bearing segmental nodes (gray circles) and outside tumor-bearing segmental nodes (open circles), according to the harbored segment. Stations 10 to 12 were considered hilar and lobar nodes (cross-hatched circles.)

These prospectively collected clinical and pathological data were retrospectively analyzed with respect to the relationships between clinicopathologic features including tumor location, cancer spread pattern in the intrapulmonary area, and cancer spread pattern outside the tumor-bearing segment.

Statistical Analysis

Frequency analysis was performed using the χ^2 test. The Wilcoxon rank sum test was applied to continuous data. Data were analyzed using JMP 10 software (SAS Institute, Cary, NC). All p values were based on a two-tailed hypothesis test; a p value of less than 0.05 was considered to have statistical significance.

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Results

Patient Characteristics

In a total of 406 lesions treated during the study period, 27 double cancers and 3 triple cancers were included. Based on the exclusion criteria, 162 lesions were omitted from the analysis for the following reasons: resection of single segment or less (n = 58), no systematic sampling or dissection (n = 9), ipsilateral multiple lesions (n = 49), small cell lung cancer and rare histological type of NSCLC (n = 12), and preoperative anticancer treatments (n = 34). Consequently, 244 lesions were subjected to the analysis described below.

The characteristics of eligible lesions are summarized in Table 1. The 244 eligible lesions were present in 170 male patients and 74 female patients, and the average patient age was 68.4 ± 8.3 years. Primary tumor locations included peripheral type (n = 167), intermediate type (n = 49), and central type (n = 28). The CT findings were pure GGO tumors (n = 20), mixed GGO tumors (n = 44), and solid-type tumors (n = 180). Tumors were located in the right lung in 138 cases and in the left lung in 106 cases. Tumor location details are shown in Figure 2. The cohort included 167 adenocarcinomas, 66 squamous cell carcinomas, and 11 large cell carcinomas. Pathologic stages included stage 0 to IA (n = 111), stage IB (n = 56), stage IIA (n = 31), stage IIB (n = 20), stage IIIA (n = 23), and IIIB to IV (n = 3). Pathologic nodal metastasis was observed in 48 lesions (20%), including 26 pN1 and 22 pN2 cases.

Table 1. Characteristics of Eligible Patients

Characteristics	All
Eligible lesions	244
Male/female	170/74
Age, years, ± SD	68.4 ± 8.3
Lesion side, right/left	138/106
Location of primary tumor	
Peripheral	167
Intermediate/central	77
Computed tomography findings of primary lesion	
Pure ground glass opacity	20
Mixed ground glass opacity	44
Solid	180
Tumor diameter, mm	
≤20	63
>20 to ≤30	76
>30	105
Average	30.7 ± 15.1
Chronic obstructive pulmonary disease lung	47
Interstitial pneumonia lung	26
Surgery	
Pneumonectomy	4
Lobectomy	210
Segmentectomy	30
Histology	
Adenocarcinoma	167
Squamous cell carcinoma	66
Large cell carcinoma	11
Nodal metastasis	48
pN0	196
pN1	26
pN2	22
Differentiation	
Grade 1, well	70
Grade 2, moderate	108
Grade 3, poor	53
Lymphatic permeation (+)	39
Vessel invasion (+)	88
Pathologic pleural invasion (+), pl 1/2/3	31/12/23

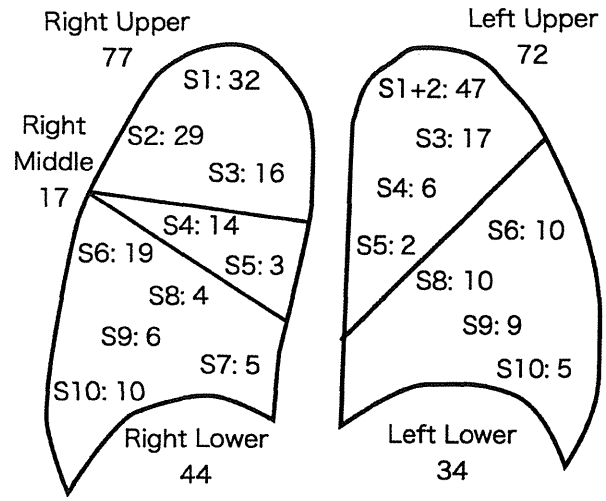

Fig 2. Primary tumor location. Primary lesions were observed in the following lobes: 77 (32%) in the right upper lobe, 17 (7%) in the right middle lobe, 44 (18%) in the right lower lobe, 72 (30%) in the left upper lobe, and 34 (14%) in the left lower lobe. (S = segment.)

Extrapulmonary and Intrapulmonary Metastasis

Among 48 cases with nodal metastasis, extrapulmonary nodal metastases were identified in 38 lesions, and occurred more frequently in lesions in intermediate/central locations (Table 2). Among the 38 lesions with extrapulmonary node metastasis, intermediate/central type and solid type lesions comprised a larger proportion than they did among the 206 lesions without extrapulmonary node metastasis. Pathologically, lymphatic permeation and vessel invasion were more frequently detected in lesions with extrapulmonary node metastasis than in lesions without such metastasis, whereas no difference in pleural invasion was observed between the two groups (Table 2).

Metastatic intrapulmonary nodes were observed in 20 lesions, representing 42% of lesions with nodal metastasis (Table 2). Intrapulmonary parenchymal metastasis (pm1) occurred in five lesions (2.0%), one of which was simultaneously accompanied by extrapulmonary nodal metastasis. All pm1 lesions occurred in tumor-bearing

Table 2. Clinical and Pathologic Factors of Cases With and Without Extrapulmonary Node Metastasis

Clinical and Pathologic Factors	All	Extrapulmonary Nodes (Hilar to Mediastinum) Stations 1–12 (+)	(−)	*p* Value
Total	244	38	206	
Intermediate/central	77	18	59	0.04
Solid appearance	180	37	143	<0.0001
Average tumor size, mm	30.7 ± 15.1	33.4 ± 14.8	30.2 ± 16.1	0.11
COPD lung	47	7	40	1.00
Interstitial pneumonia lung	26	3	23	0.78
Intrapulmonary nodes (stations 13, 14)	20	11	9	<0.0001
Parenchymal metastasis (pm1)	5	1	4	0.57
Intrapulmonary spread (stations 13, 14, or pm1)	24	12	12	<0.0001
Metastasis inside tumor-bearing segment (stations 13, 14, pm1)	20	9	11	0.001
Metastasis outside tumor-bearing segment (stations 13, 14, pm1)	4	3	1	0.001
Lymphatic permeation (+)	39	18	21	<0.0001
Vessel invasion (+)	88	25	63	<0.0001
Any pathologic pleural invasion (+)	66	14	52	0.17

COPD = chronic obstructive pulmonary disease.

segments. These five lesions were poorly to moderately differentiated adenocarcinomas. Altogether, intrapulmonary spread was observed in 24 lesions (9.8%), of which 19 were segmental/intersegmental nodal metastases, 4 were parenchymal metastases, and 1 was both. Of the 24 lesions, 12 (50%) were associated with extrapulmonary node metastasis, including 3 instances of skip N2 metastasis. Intrapulmonary spread was more frequently observed in lesions with extrapulmonary node metastasis (Table 2), intermediate/central type tumors, and in solid type lesions compared with their respective counterparts (Tables 2 and 3).

Metastasis outside the tumor-bearing segment was observed in only 4 cases (17%), representing only 1.6% all lesions (Table 2). Three of the four lesions were associated with extrapulmonary node metastasis, representing 7.9% of lesions (3 of 38) with extrapulmonary node metastasis (Table 2); the other one was not associated with any nodal metastasis or parenchymal metastasis, and represented only 0.5% of the total lesions (1 of 206) without extrapulmonary metastasis. Among all lesions, primary tumors were most commonly solid type, whereas primary tumor locations were not significantly associated with the incidence of the type of metastasis (Table 3). In the 4 cases with metastasis outside the tumor-bearing segment, interestingly, all primary tumors were located in the left lung, three being in the left upper lobe.

Peripheral Small Tumors

Of the 167 peripheral type lesions, 52 (31%) were 20 mm or less in diameter. Among these, nodal metastasis was observed in 9.6% of lesions (5 of 52), all of which were solid type and were associated with multiple nodal

Table 3. Tumor Localization, Computed Tomography Findings, and Intrapulmonary Spread

Characteristics	All	Peripheral	Intermediate/ Central	*p* Value	Pure/Mixed GGO	Solid	*p* Value
Total	244	167	77		64	180	
Extrapulmonary nodes (stations 1–12)	38	20	18	0.04	1	37	<0.0001
Hilar and lobar nodes (stations 10–12)	30	15	15	0.03	1	29	0.0014
Skip N2	8	5	3	0.71	0	8	0.12
Intrapulmonary nodes (stations 13, 14)	20	9	11	0.02	1	19	0.03
Parenchymal metastasis (pm1)	5	1	4	0.04	0	5	0.33
Intrapulmonary spread (stations 13, 14, or pm1)	24	10	14	0.005	1	23	0.007
Metastasis inside tumor-bearing segment (stations 13, 14, pm1)	20	8	12	0.01	1	19	0.03
Metastasis outside tumor-bearing segment (stations 13, 14, pm1)	4	2	2	0.59	0	4	0.57

GGO = ground glass opacity.

metastasis in extrapulmonary nodes. Spread outside tumor-bearing segments was not observed, whereas spread inside tumor-bearing segments was observed in 1 case (1.4%). In lesions without extrapulmonary node metastasis, no intrapulmonary spread was observed in association with peripheral small (≤20 mm) tumors. Among the 167 peripheral type lesions, 103 (62%) had a diameter of 30 mm or less. Nodal metastasis was observed in 10 of these 103 lesions (9.7%), all of which were solid type primary tumors.

Tumor Spread Pattern

A flowchart based on tumor-spread pattern is illustrated in Figure 3. Cancer spread outside tumor-bearing segments in patients with hilar or mediastinal nodal metastasis occurred in 7.9% cases (3 of 38), whereas the frequency was 0.5% (1 of 206) among patients without hilar or mediastinal nodal metastasis; this result is consistent with the concept of the bronchopulmonary segment. As such, the 205 lesions might be completely resected by segmentectomy following the right path in Figure 3. The extrapulmonary node metastasis is searchable at the time of surgery; therefore, this would be a crucial condition for selection of candidates for segmentectomy. Among these 205 lesions, 194 lesions without intrapulmonary spread might be radically removed by wedge resection; however, such metastasis can only be demonstrated through detailed pathologic examination. There would be no doubt that lobectomy or more substantial surgery should be used in the 38 cases with extrapulmonary nodal metastases (left half of the path of Fig 3). However, 35 of the 38 cases (92%) were not accompanied by spread outside the tumor-bearing segment.

Comment

It is very important to better understand intrapulmonary spread pattern of NSCLC because spread to outside of tumor-bearing segment leads to locoregional recurrence in case of segmentectomy; that is now paid great attention as a novel surgical modality for peripheral small NSCLC. In case no lymph node metastases are found in resected specimens, patients would not undergo any treatments until local recurrence appears even if they have metastasis outside of tumor-bearing segments. The historical randomized trial conducted by the Lung Cancer Study Group [4] clearly indicated that local recurrence after sublobar resection is associated with unfavorable survival. In this study, the intrapulmonary spread pattern of NSCLC was fully examined using resected specimens.

Nodal metastasis to the intrapulmonary region is a common pattern of spread, and nodal metastasis to stations 12 and 13 has been reported to occur in 38.5% of pN1 patients [17]. Furthermore, the prognosis of pN1 patients with only intrapulmonary nodal metastasis (ie, stations 13 and 14) has been reported to be as poor as that for pN1 patients without intrapulmonary nodal metastasis [18]. Thus, the indication for limited resection should be carefully defined to avoid failure in removal of intrapulmonary metastatic lesions. In particular, metastasis outside tumor-bearing segments is an issue to be resolved. In this study, we analyzed the intrapulmonary spread pattern by nodal and parenchymal inspection of resected lungs; metastasis outside tumor-bearing segments was observed in only four lesions (1.6% of total lesions), and the clinicopathologic features of these lesions included primary tumor in the left upper lobe (75%), solid tumor (100%), and multiple nodal metastases in extrapulmonary nodes (75%).

We observed one lesion located in the superior lingular segment (S4) with intrapulmonary metastasis outside the tumor-bearing segment that was not associated with any other metastasis. Topol and associates [19] demonstrated the existence of a nodal metastatic tract across the intersegmental plane between the superior and inferior lingular segments in 2 of 135 examinations of cadaveric lungs. The tract may function as another lymphatic metastatic route. Even though this tract is very rare, it should be kept in mind when considering sublobar resection. Another explanation for this lesion not being associated with any other metastasis is remodeling of lymphatics due to chronic lung disease [20], as the patient had chronic obstructive pulmonary disease and interstitial pneumonia.

Solid type tumors comprise 96% of lesions (23 of 24) with intrapulmonary metastasis, 100% (5 of 5) with parenchymal spread, and 100% (4 of 4) with metastasis

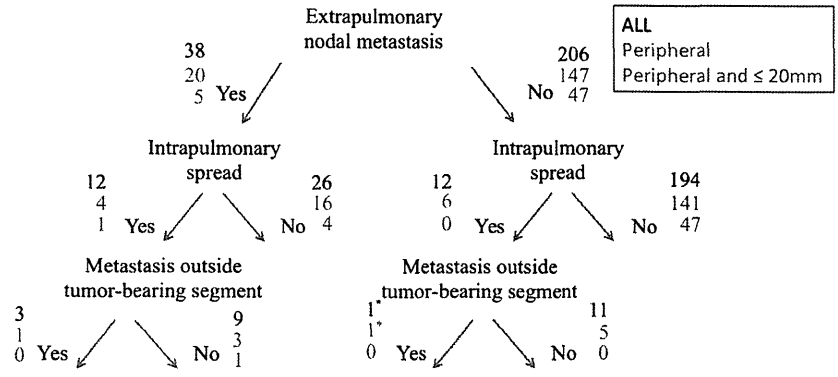

Fig 3. Tumor spread pattern. The algorithm shows a cascade based on three tumor-spread processes: (1) extrapulmonary nodal metastasis, (2) intrapulmonary spread, and (3) metastasis outside tumor-bearing segments. The number of lesions that fulfill each condition are shown, from the top: total lesions (black numbers), peripheral lesions (green numbers), and 20 mm or less peripheral lesions (red numbers). The single patient with metastasis on the outside of the tumor-bearing segment without any hilar or mediastinal nodal metastasis is noted by an asterisk (*).

outside tumor-bearing segments. In the literature, 16% of solid type tumors of 20 mm or less are associated with nodal metastasis [21]. Consequently, solid tumors should be carefully considered for limited surgery.

Small tumor size should be a required condition for limited surgery in consideration of surgical margins. Okada and associates [22] suggested segmentectomy with lymph node assessment as an alternative to lobectomy in patients with 20 mm or less NSCLC, and the present results support this theory. Several studies have advocated that candidates include a tumor size of 30 mm or less [7, 9, 22]. In the present study, of 103 lesions with a diameter of 30 mm or less, 3 intrapulmonary spread lesions (2.9%) without any extrapulmonary nodal metastasis were observed. In contrast, no such lesions were observed among 52 lesions with a diameter of 20 mm or less. Thus, nonanatomic sublobar resection for peripheral tumors 30 mm or less could allow the tumor to remain. In our series, 5 patients of 27 with mixed GGO tumors more than 20 mm underwent segmentectomy owing to inadequate pulmonary reserve. Recently, Asamura and colleagues [23] reported that 2 cm to 3 cm (T1b) adenocarcinoma with 0.5 or less consolidation/tumor diameter ratio showed a significantly favorable prognosis, with 96.4% survival at 5 years after lobectomy in a prospective observational study (JCOG0201). Such tumors may be well indicated for sublobar resection. Now the Japanese Clinical Oncology Group plans to start a new prospective study to verify this issue.

Skipping N2 metastasis, which occurred in 8 cases (3.3%), must also be considered. No bias was observed with regard to the side of the lung or tumor location; however, it was noted for peripheral small tumors (38% in tumors ≤20 mm, and 50% in tumors ≤30 mm). In the literature, this type of nodal spread has been reported to occur in 20% to 40% of patients, as detected during autopsy [24], as well as in 35% of N2 patients [25]. Based on these observations, we assume that the skip N2 pattern must be approached carefully when performing limited surgery. In addition to hilar and lobar node inspection, mediastinal nodal inspection must be conducted even when limited resection is performed as accurate nodal staging is crucial to the decision regarding adjuvant chemotherapy.

Finally, the optimal candidacy for limited resection, particularly segmentectomy, must be addressed. Development of strategies for judging the presence of such metastasis might increase the number of candidates for segmentectomy. Computed tomography is one of the tools used to select candidates for segmentectomy, as reported previously [26]. In 64 pure/mixed GGO type lesions in this study, no spread outside the tumor-bearing segment was observed, whereas cancer spread inside the tumor-bearing segment was seen in one lesion. In 52 peripheral small size (≤20 mm) tumors without extrapulmonary nodal metastasis, no intrapulmonary metastasis was found; therefore, such lesions also appear to be good candidates for segmentectomy from the viewpoint of intrapulmonary spread pattern.

In conclusion, cancer spread to the outside of the tumor-bearing segment is infrequently observed in lung cancers when the tumor is not associated with extrapulmonary nodal metastasis, in pure-mixed GGO lesions or in peripheral small (≤20 mm) lesions.

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References

1. Cahan WG. Radical lobectomy. J Thorac Cardiovasc Surg 1960;39:555–72.
2. Jensik RJ, Faber LP, Milloy FJ, Monson DO. Segmental resection for lung cancer. A fifteen-year experience. J Thorac Cardiovasc Surg 1973;66:563–72.
3. Errett LE, Wilson J, Chiu RC, Munro DD. Wedge resection as an alternative procedure for peripheral bronchogenic carcinoma in poor-risk patients. J Thorac Cardiovasc Surg 1985;90:656–61.
4. Ginsberg RJ, Rubinstein LV. Randomized trial of lobectomy versus limited resection for T1 N0 non-small cell lung cancer. Lung Cancer Study Group. Ann Thorac Surg 1995;60:615–22.
5. Landreneau RJ, Sugarbaker DJ, Mack MJ, et al. Wedge resection versus lobectomy for stage I (T1 N0 M0) non-small-cell lung cancer. J Thorac Cardiovasc Surg 1997;113: 691–8.
6. El-Sherif A, Gooding WE, Santos R, et al. Outcomes of sublobar resection versus lobectomy for stage I non-small cell lung cancer: a 13-year analysis. Ann Thorac Surg 2006;82:408–15.
7. Kodama K, Doi O, Higashiyama M, Yokouchi H. Intentional limited resection for selected patients with T1 N0 M0 non-small-cell lung cancer: a single-institution study. J Thorac Cardiovasc Surg 1997;114:347–53.
8. Okada M, Koike T, Higashiyama M, Yamato Y, Kodama K, Tsubota N. Radical sublobar resection for small-sized non-small cell lung cancer: a multicenter study. J Thorac Cardiovasc Surg 2006;132:769–75.
9. Tsutani Y, Miyata Y, Nakayama H, et al. Oncologic outcomes of segmentectomy compared with lobectomy for clinical stage IA lung adenocarcinoma: propensity score-matched analysis in a multicenter study. J Thorac Cardiovasc Surg 2013;146:358–64.
10. Churchill ED, Belsey R. Segmental pneumonectomy in bronchiectasis: the lingula segment of the left upper lobe. Ann Surg 1939;109:481–99.
11. Foster-Carter AF, Hoyle C. The segments of the lungs; a commentary on their investigation and morbid radiology. Dis Chest 1945;11:511–64.
12. Colombano SP, Reese PA. The cascade theory of metastatic spread: are there generalizing sites? Cancer 1980;46:2312–4.
13. Fraser RG. Diagnosis of diseases of the chest. Philadelphia: Saunders; 1978:1117–32.
14. Saeteng S, Tantraworasin A, Euathrongchit J, Lertprasertsuke N, Wannasopha Y. Nodal involvement pattern in resectable lung cancer according to tumor location. Cancer Manage Res 2012;4:151–8.
15. Yoshino I, Nakanishi R, Osaki T, et al. Postoperative prognosis in patients with non-small cell lung cancer with synchronous ipsilateral intrapulmonary metastasis. Ann Thorac Surg 1997;64:809–13.
16. Rusch VW, Asamura H, Watanabe H, et al. The IASLC lung cancer staging project: a proposal for a new international lymph node map in the forthcoming seventh edition of the TNM classification for lung cancer. J Thorac Oncol 2009;4: 568–77.

17. Yano T, Yokoyama H, Inoue T, Asoh H, Tayama K, Ichinose Y. Surgical results and prognostic factors of pathologic N1 disease in non-small-cell carcinoma of the lung. Significance of N1 level: lobar or hilar nodes. J Thorac Cardiovasc Surg 1994;107:1398–402.
18. Maeshima AM, Tsuta K, Asamura H, Tsuda H. Prognostic implication of metastasis limited to segmental (level 13) and/or subsegmental (level 14) lymph nodes in patients with surgically resected nonsmall cell lung carcinoma and pathologic N1 lymph node status. Cancer 2012;118:4512–8.
19. Topol M, Maslon A. Some variations in lymphatic drainage of selected bronchopulmonary segments in human lungs. Ann Anat 2009;191:568–74.
20. Hardavella G, Tzortzaki EG, Siozopoulou V, et al. Lymphangiogenesis in COPD: another link in the pathogenesis of the disease. Respir Med 2012;106:687–93.
21. Hattori A, Suzuki K, Matsunaga T, et al. Is limited resection appropriate for radiologically "solid" tumors in small lung cancers? Ann Thorac Surg 2012;94:212–5.
22. Okada M, Yoshikawa K, Hatta T, Tsubota N. Is segmentectomy with lymph node assessment an alternative to lobectomy for non-small cell lung cancer of 2 cm or smaller? Ann Thorac Surg 2001;71:956–60.
23. Asamura H, Hishida T, Suzuki K, et al. Radiologic determined noninvasive adenocarcinoma of the lung: survival outcomes of Japan Clinical Oncology Group 0201. J Thorac Cardiovasc Surg 2013;146:24–30.
24. Riquet M. Anatomic basis of lymphatic spread from carcinoma of the lung to the mediastinum: surgical and prognostic implications. Surg Radiol Anat 1993;15:271–7.
25. Yoshino I, Yokoyama H, Yano T, et al. Skip metastasis to mediastinal nodes in non-small cell lung cancer. Ann Thorac Surg 1996;62:1021–5.
26. Suzuki K, Koike T, Asakawa T, et al, for the Japan Lung Cancer Surgical Study Group (JCOG LCSSG). A prospective radiological study of thin-section computed tomography to predict pathological noninvasiveness in peripheral clinical IA lung cancer (Japan Clinical Oncology Group 0201). J Thorac Oncol 2011;6:751–6.

INVITED COMMENTARY

The work of Sakairi and associates [1] provokes thoughts on our basic concepts of "adequacy of surgery" fostered from the Halstedian "centrifugal pattern" of cancer spread [2]. Although Halsted's "complete surgical management" concept was challenged even after its inception [3], this deeply rooted dogma of our surgical heritage is a hard principle from which to depart.

The authors provide rather convincing data carefully assimilated from a large clinicopathologic evaluation of anatomic resections of 2 or more pulmonary segments for what I perceive was believed to be clinical stage I primary lung cancer amenable to sublobar resection. They conclude that the likelihood of tumor spread beyond the confines of the parenchyma of the "extended segmentectomies" of this series was very unlikely.

Among patients with small peripheral lesions (< 2.0 cm in diameter) or with predominant ground-glass lesions by computed tomographic imaging, the occurrence was negligible. However, the occurrence was greatest among those patients found on pathologic review to have had extrapulmonary nodal metastases (15.5% of the entire group).

I come away with a few thoughts and further questions regarding this analysis. It appears that the primary determining factors related to reliance on segmentectomy as definitive local therapy for clinical stage I lung cancer are tumor size and morphologic characteristics of the lesion seen on computed tomography. The primary negative determinant is the identification of intraoperative determination of extraparenchymal nodal metastases.

The similarity of these findings to those of sentinel node evaluation for stage I and stage II breast cancer in determining the use of axillary lymph node dissection is striking. Because no clinical outcomes related to these anatomic/pathologic findings are provided in this work, we conjecture that the use of more radical surgical procedures for a positive "sentinel node" finding may have the equivocal long-term outcome as noted with lesser surgical procedures for breast cancer [4].

Although local failure can be an important consideration for the minority of patients undergoing anatomic segmentectomy for presumed favorable peripheral stage I lung cancers, these recurrences and the presence of nodal metastases are largely harbingers of an aggressive phenotype of disease beyond the boundaries of the surgeon's knife.

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References

1. Sakairi Y, Yoshino I, Yoshida S, et al. Patterns of metastasis outside tumor-bearing segments in primary lung cancer: rationale for segmentectomy. Ann Thorac Surg 2014;97:1694–700.
2. Halsted WS. The results of radical operations for the cure of carcinoma of the breast. Ann Surg 2007;46:1–19.
3. Criles G Jr. The evolution of the treatment of breast cancer. In: Wise L, Johnson Jr H, eds. Breast Cancer: Controversies in Management. Armonk, NY: Futura Publishing; 1994.
4. Yi M, Giordano SH, Meric-Bernstam F, et al. Trends in and outcomes from sentinel lymph node biopsy (SNLB) alone vs. SNLB with axillary node dissection for node-positive breast cancer patients: experience with the SEERS database. Ann Surg Oncol 2010;17:343–51.

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Original Study

Clinicopathological Features in Young Patients Treated for Small-Cell Lung Cancer: Significance of Immunohistological and Molecular Analyses

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Abstract

The validity of the diagnosis in young patients who had been diagnosed as having small-cell lung cancer (SCLC) has not been adequately described. We reevaluated the clinical data of 8 young patients. Genetic rearrangements of nuclear protein of the testis (*NUT*) were revealed in 2 patients. Caution is needed when diagnosing SCLC, especially in young patients.

Background: Small-cell lung cancer in young patients is very rare and has not been adequately described. In addition, malignancies associated with genetic rearrangements of nuclear protein of the testis (*NUT*) have been reported in young patients. **Patients and Methods:** We reviewed the clinical records of patients younger than 40 years of age who had been diagnosed as having SCLC and had been treated for this condition. We also examined *NUT* rearrangements using immunohistochemistry (IHC) staining and fluorescence in situ hybridization (FISH) analysis. **Results:** We evaluated the diagnoses and treatment outcomes of 8 young patients among 747 SCLC patients. Based on further analyses using IHC staining and FISH, *NUT* rearrangements were found in 2 of these cases. The range of the overall survival period was 3.6 to 49.7 months. The 2 patients with *NUT* rearrangements survived for less than 12 months. **Conclusion:** *NUT* rearrangements were identified in 2 patients who had been previously diagnosed as having SCLC. Further attention regarding the diagnosis of SCLC in young patients is needed.

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Introduction

The median age at the time of the diagnosis of lung cancer is 71 years according to the Surveillance, Epidemiology and End Results Cancer Statistics. Lung cancer in patients younger than the age of 40 years is rare and comprises approximately 2.7% of all lung cancers.[1] Various reports have discussed the prognosis of lung cancer in young patients. Some studies have shown that young patients have a better prognosis,[2,3] and others have reported no survival differences between young and old patients.[1,4]

Small-cell lung cancer (SCLC) is an undifferentiated neoplasm composed of primitive-appearing small cells, and rapid progression and extensive metastases are typically observed at the time of presentation. Some previous articles have reported the incidence of SCLC in young patients.[1,4-8] SCLC patients account for 0% to 5% of lung cancer patients younger than 40 years of age.[1,4] However, the treatment outcomes have not been reported and the results of the pathological examinations have not been validated in young SCLC patients.

Recently, carcinomas with nuclear protein of the testis (*NUT*) rearrangements have been included in the differential diagnosis of SCLC because of their morphological similarities. *NUT* midline carcinoma (NMC) often arises from midline structures, such as the mediastinum and the upper aerodigestive tract, in young people. NMC is a rare and aggressive carcinoma that is characterized by chromosomal rearrangement at the *NUT* gene.[9] NMC is a lethal

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disease despite intensive therapies[10,11] and must be considered in differential diagnoses of poorly differentiated squamous cell carcinoma, undifferentiated carcinoma, and other small round cell tumors.[12] SCLC with *NUT* rearrangements has not been previously reported.

The objective of the present study was to reevaluate the validity of the diagnosis of SCLC in young patients before the era of immunohistochemistry (IHC) staining and molecular analyses, including the evaluation of *NUT* rearrangements. We also evaluated the clinical response to treatment and the outcome of SCLC in young patients.

Patients and Methods

Patients

Small-cell lung cancer patients who were 40 years old or younger and who had been treated with chemotherapy at the National Cancer Center Hospital in Tokyo, Japan, between 1993 and 2010 were retrospectively identified.

Data Collection and Evaluation of Tumor Response

The following clinical data were collected from the medical records: patient characteristics, treatment regimens, and treatment outcomes. The tumor responses were evaluated according to the Response Evaluation Criteria in Solid Tumors, version 1.1. We evaluated the best overall response. When the disease status was stably maintained for more than 8 weeks, the patient was considered to have stable disease.

Immunohistochemical and Molecular Analyses

For IHC staining, 4-μm thick sections from a paraffin block were routinely deparaffinized. The detailed antigen retrieval methods and antibody dilutions used for each primary antibody are listed in Table 1. We used an automated stainer (DAKO, Carpinteria, CA) for the primary antibody incubation, according to the vendor's protocol. ChemMateEnVision (DAKO) detection methods were used.

To assess the presence of *NUT* rearrangements, we used break-apart *NUT* probes (RP11-412E10 for *NUT* centromere and RP11-1H8 for *NUT* telomere; Chromosome Science Lab, Inc, Sapporo, Japan) according to the manufacturer's instructions. At least 50 nonoverlapping tumor cells were examined, and cases with more than 20% of the cells showing split-apart signals were considered to be positive for *NUT* rearrangements.

Survival Definition

Overall survival was defined as the period between the start of the first treatment and death from any cause or the last follow-up examination.

Results

Patient Characteristics

A retrospective review of 747 patients who had been diagnosed as having SCLC was conducted. Although 9 patients younger than the age of 40 years were originally diagnosed as having SCLC and were treated accordingly, the tumor in 1 patient did not exhibit the typical morphological features of SCLC according to the presently used pathological criteria. Thus, we excluded this patient and ultimately retrieved clinical data for 8 patients (1.1%) younger than the age of 40 years. The patients were between the ages of 18 and 40 and consisted of 4 men and 4 women; 5 of the patients were current smokers. Three patients had limited disease SCLC (LD-SCLC), and 5 patients had extended disease SCLC.

Histological Profiles

Among the 8 cases, 4 cases were reevaluated using hematoxylin and eosin (H & E) and IHC staining. The other 4 cases were reviewed based on pathological reports obtained from the primary hospital. Based on the standard pathological criteria used for the diagnosis of SCLC,[13] 4 patients had received accurate diagnoses of SCLC (patients 1-4). However, the 4 other patients might not have actually had SCLC, because these patients exhibited atypical morphological features for SCLC (patients 5-8). The clinical information and the IHC results for all patients are listed in Table 2. *NUT* rearrangements were observed in 2 patients (Patients 7 and 8). One patient (patient 7) had positive *NUT* IHC and fluorescence in situ hybridization findings in addition to exhibiting the typical morphological features of SCLC (Fig. 1).

Clinical Response and Outcome

Overall, 4 of the 8 patients responded to first-line treatment (4 partial response, 2 stable disease, 1 progressive disease, and 1 not evaluated). All 3 LD-SCLC patients had partial responses to chemoradiotherapy. Of the 2 NMC patients, 1 NMC patient (patient 7) had progressive disease after 2 cycles of cisplatin-based chemotherapy. Another NMC patient (patient 8) had a partial response to 2 cycles of cisplatin-based chemotherapy. The overall survival periods of the patients ranged from 3.6 to 49.7 months. The patients with *NUT* rearrangements survived for less than 12 months.

Discussion

In our study, we used immunohistological and molecular analyses to reevaluate the treatment outcomes and the validity of the diagnoses in young patients who had been diagnosed as having SCLC. Based on our reevaluation of 8 patients, we could identify only

Table 1 Antibodies Used for the Immunohistochemical Analysis

Antibody	Source	Clone	Pretreatment	Dilution
TTF-1	DAKO	8G7G3/1	Citrate buffer	1/100
CD56	Novocastra	1B6	Citrate buffer	1/200
CD99	SIGNET	013	Citrate buffer	1/50
Synaptophysin	DAKO	27G12	TRS9 (98°C, 40 min)	1/100
Chromogranin A	DAKO	—	Citrate buffer	1/500
NUT	Cell Signaling	C52B1	TRS9 (98°C, 40 min)	1/45

Table 2 Patient Characteristics and Histological Profiles

Patient	Sex	Age	PS	Stage	ProGRP	IHC Results	CD99	Initial Treatment	Response	OS, Months
1	F	18	0	LD	657	+	–	Chemoradiotherapy	PR	49.7
2	M	40	1	LD	ND	+	–	Chemoradiotherapy	PR	19.7
3	M	39	1	ED	11,040	ND	ND	Chemotherapy alone	SD	12.3
4	F	34	1	ED	12,010	+	ND	Chemotherapy alone	SD	43.1
5	M	39	1	LD	ND	ND	ND	Chemoradiotherapy	PR	18.8
6	F	21	1	ED	ND	–	ND	Chemotherapy alone	NE	6.0
7	M	24	1	ED	38	+[a]	–	Chemotherapy alone	PD	7.2
8	F	29	1	ED	28	–	–	Chemotherapy alone	PR	3.6

Abbreviations: ED = extended disease; F = female; IHC results = immunohistochemistry results for neuroendocrine antigens; LD = limited disease; M = male; ND = no available data; NE = not evaluated; OS = overall survival; ProGRP = progastrin-releasing peptide; PS = performance status.
[a]CD56 was positive in only part of the tumor.

4 patients who had received accurate diagnoses of SCLC. Evaluations based only on morphological features were likely to have resulted in misdiagnosis; 2 of the patients were ultimately diagnosed as having NMC. Thus, special attention to the possible presence of NMC mimicking SCLC is needed for the differential diagnosis of SCLC in patients younger than 40 years.

Most SCLCs exhibit a typical morphology and their diagnosis is thus straightforward, with IHC staining being unnecessary.[13] However, in problematic cases, such as in young patients, nonsmokers, and tumors that are difficult to distinguish from other malignancies, a diagnosis of SCLC should be very carefully performed using immunohistochemical and molecular diagnostic techniques. Of note, our additional analyses revealed the presence of NMCs in 2 of the patients who had originally been diagnosed as having SCLC and had been treated accordingly. Thus, for the accurate diagnosis of SCLC, especially in young patients, not only light microscopy examinations but also immunohistochemical and molecular analyses should be performed.

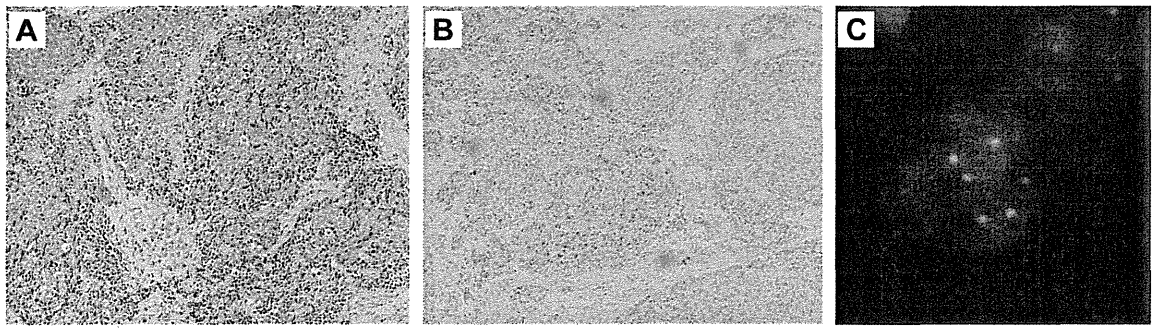
Approximately 60 cases of NMC have been reported to date.[14] In two-thirds of these reported NMC cases, *NUT* on chromosome 15q14 is fused to *BRD4* on chromosome 19p13.1, forming *NUT-BRD4*. In approximately one-third of the cases, the partner gene is *BRD3* or an uncharacterized gene (*NUT-BRD3* and *NUT* variants).[10,15] The histological differential diagnosis of NMC includes poorly differentiated squamous cell carcinoma, undifferentiated carcinoma, and other small blue round cell tumors, such as primitive neuroectodermal tumor.[12] In our study, the H & E results in 2 patients with NUT rearrangements showed features that were consistent with combined small-cell and squamous cell carcinoma in 1 patient (patient 7) and with small-cell carcinoma in another patient (patient 8). Previous reports and review articles have suggested that negative neuroendocrine antigen results are helpful for the diagnosis of NMC[12]; however, patient 7 exhibited not only a neuroendocrine morphology, but also immunopositivity for CD56. Although CD56 is not a complete marker for neuroendocrine differentiation, NMC with positive neuroendocrine markers has not been previously reported; thus, this is the first case report to describe such a lesion. These results indicate that young patients should not be diagnosed as having SCLC based only on a neuroendocrine morphology and a neuroendocrine phenotype without performing an analysis to detect *NUT* rearrangements.

Concerning the treatment of NMC, most previously reported patients received combination multidrug chemotherapy, such as platinum-based regimens[16,17] and lymphoma regimens[16]; however, most of these patients died within 1 year.[10,15] No drugs that contribute to a long survival period have been found. As for new agents, the domain inhibitor for *BRD4* and histone deacetylase inhibitors have been studied and reported in some journals.[18-20] Although these agents are still in development, the use of these agents for the treatment of NMC is anticipated. The accumulation of numerous NMC cases is important, and the development of more effective treatments for patient with NMC is needed.

Conclusion

Malignancies in young patients should be carefully diagnosed using IHC and molecular diagnostic techniques. Moreover, the possibility of NMC should be considered, especially in young

Figure 1 Histologic Features. (A) Dense Sheets of Small Cells With Granular Nuclear Chromatin are Visible on Hematoxylin and Eosin Staining in Patient 7. (B) Immunohistochemistry and (C) Fluorescence in Situ Hybridization Findings Were Positive for *NUT*



patients thought to have SCLC in which atypical histological features are observed using H & E and IHC staining.

Clinical Practice Points

- Most SCLC cases can be diagnosed using H & E staining.
- However, in problematic cases, such as young patients, nonsmokers, and tumors that are difficult to distinguish from other malignancies, the diagnosis of SCLC should be very carefully performed.
- In our study, genetic rearrangement of *NUT* was revealed in 2 patients.
- We suggest that a diagnosis of SCLC should be very carefully performed using immunohistochemical and molecular diagnostic techniques, especially in young patients.

Disclosure

The authors have stated that they have no conflicts of interest.

References

1. Sekine I, Nishiwaki Y, Yokose T, Nagai K, Suzuki K, Kodama T. Young lung cancer patients in Japan: different characteristics between the sexes. *Ann Thorac Surg* 1999; 67:1451-5.
2. Radzikowska E, Roszkowski K, Glaz P. Lung cancer in patients under 50 years old. *Lung Cancer* 2001; 33:203-11.
3. Ramalingam S, Pawlish K, Gadgeel S, Demers R, Kalemkerian GP. Lung cancer in young patients: analysis of a Surveillance, Epidemiology, and End Results database. *J Clin Oncol* 1998; 16:651-7.
4. Maruyama R, Yoshino I, Yohena T, et al. Lung cancer in patients younger than 40 years of age. *J Surg Oncol* 2001; 77:208-12.
5. Jiang W, Kang Y, Shi GY, et al. Comparisons of multiple characteristics between young and old lung cancer patients. *Chin Med J (Engl)* 2012; 125:72-80.
6. Yazgan S, Gursoy S, Yaldiz S, Basok O. Outcome of surgery for lung cancer in young and elderly patients. *Surg Today* 2005; 35:823-7.
7. Tian DL, Liu HX, Zhang L, et al. Surgery for young patients with lung cancer. *Lung Cancer* 2003; 42:215-20.
8. Yu DC, Grabowski MJ, Kozakewich HP, et al. Primary lung tumors in children and adolescents: a 90-year experience. *J Pediatr Surg* 2010; 45:1090-5.
9. French CA, Miyoshi I, Kubonishi I, Grier HE, Perez-Atayde AR, Fletcher JA. *BRD4-NUT* fusion oncogene: a novel mechanism in aggressive carcinoma. *Cancer Res* 2003; 63:304-7.
10. Stelow EB. A review of *NUT* midline carcinoma. *Head Neck Pathol* 2011; 5:31-5.
11. French CA, Kutok JL, Faquin WC, et al. Midline carcinoma of children and young adults with *NUT* rearrangement. *J Clin Oncol* 2004; 22:4135-9.
12. Stelow EB, French CA. Carcinomas of the upper aerodigestive tract with rearrangement of the nuclear protein of the testis (*NUT*) gene (*NUT* midline carcinomas). *Adv Anat Pathol* 2009; 16:92-6.
13. Travis WD. Update on small cell carcinoma and its differentiation from squamous cell carcinoma and other non–small-cell carcinomas. *Mod Pathol* 2012; 25(suppl 1):S18-30.
14. Bauer DE, Mitchell CM, Strait KM, et al. Clinicopathologic features and long-term outcomes of *NUT* midline carcinoma. *Clin Cancer Res* 2012; 18:5773-9.
15. French CA. Demystified molecular pathology of *NUT* midline carcinomas. *J Clin Pathol* 2010; 63:492-6.
16. Kubonishi I, Takehara N, Iwata J, et al. Novel t(15;19)(q15;p13) chromosome abnormality in a thymic carcinoma. *Cancer Res* 1991; 51:3327-8.
17. Vargas SO, French CA, Faul PN, et al. Upper respiratory tract carcinoma with chromosomal translocation 15;19: evidence for a distinct disease entity of young patients with a rapidly fatal course. *Cancer* 2001; 92:1195-203.
18. Filippakopoulos P, Qi J, Picaud S, et al. Selective inhibition of BET bromodomains. *Nature* 2010; 468:1067-73.
19. Delmore JE, Issa GC, Lemieux ME, et al. BET bromodomain inhibition as a therapeutic strategy to target c-Myc. *Cell* 2011; 146:904-17.
20. Schwartz BE, Hofer MD, Lemieux ME, et al. Differentiation of *NUT* midline carcinoma by epigenomic reprogramming. *Cancer Res* 2011; 71:2686-96.

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Molecular profiling of small cell lung cancer in a Japanese cohort

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ABSTRACT

Objectives: Advances in the molecular profiling of lung adenocarcinoma over the past decade have led to a paradigm shift in its diagnosis and treatment. However, there are very few reports on the molecular profiles of small cell lung cancers (SCLCs). We therefore conducted the present Shizuoka Lung Cancer Mutation Study to analyze genomic aberrations in patients with thoracic malignancies.
Materials and methods: We collected samples of SCLC from a biobank system and analyzed their molecular profiles. We assessed 23 mutations in nine genes (*EGFR*, *KRAS*, *BRAF*, *PIK3CA*, *NRAS*, *MEK1*, *AKT1*, *PTEN*, and *HER2*) using pyrosequencing plus capillary electrophoresis. We also amplified *EGFR*, *MET*, *PIK3CA*, *FGFR1*, and *FGFR2* using quantitative real-time polymerase chain reaction (PCR) and the fusion genes *ALK*, *ROS1*, and *RET* using reverse transcription PCR.
Results: Between July 2011 and January 2013, 60 SCLC patients were enrolled in the study. Samples included eight surgically resected snap-frozen samples, 50 formalin-fixed paraffin-embedded samples, and seven pleural effusion samples. We detected 13 genomic aberrations in nine cases (15%), including an *EGFR* mutation ($n = 1$, G719A), a *KRAS* mutation ($n = 1$, G12D), *PIK3CA* mutations ($n = 3$, E542K, E545K, E545Q), an *AKT1* mutation ($n = 1$, E17K), a *MET* amplification ($n = 1$), and *PIK3CA* amplifications ($n = 6$). *EGFR* and *KRAS* mutations were found in patients with combined SCLC and adenocarcinoma. No significant differences were detected in the characteristics of patients with and without genomic aberrations. However, serum neuron-specific enolase and progastrin-releasing peptide levels were significantly higher in patients without genomic aberrations than in those with aberrations ($p = 0.01$ and 0.04, respectively).
Conclusion: Genomic aberrations were found in 15% SCLC patients, with *PIK3CA* amplifications most frequently observed. To further our understanding of the molecular profiles of SCLC, comprehensive mutational analyses should be conducted using massive parallel sequencing.
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1. Introduction

Lung cancer is the most common cause of cancer-related deaths, and small cell lung cancer (SCLC) accounts for approximately 12% of all lung cancers [1]. It follows a very aggressive course, with approximately 60–70% patients having disseminated disease at diagnosis. Although SCLC shows high sensitivity to chemotherapy and radiotherapy, the median survival time for extended-disease SCLC is 8–13 months, and the 2-year survival rate is only 5% [2].

Molecular abnormalities have been discovered in patients with non-SCLC over the last decade, and these discoveries have led to a paradigm shift in its diagnosis and treatment. For example, a relationship between activating epidermal growth factor receptor (*EGFR*) mutations and response to gefitinib was reported in 2004 [3,4]. Subsequently, a number of randomized studies showed that patients with activating *EGFR* mutations were highly responsive to

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EGFR tyrosine kinase inhibitors such as gefitinib and erlotinib [5–8]. Currently, it is essential that lung adenocarcinomas are classified on the basis of genomic aberrations to ensure that patients are treated with the appropriate molecular-targeted drugs [9,10]. Analyses of genomic aberrations and the development of new molecular-targeted drugs are ongoing for lung adenocarcinoma. In contrast, there have been few innovations in the treatment of SCLC, despite extensive basic and clinical research over the past 30 years.

There have been few molecular profiles of SCLC, and, till date, no molecular-targeted drugs have shown clinical activity against SCLC [11]. Identification of genomic aberrations linked to SCLC would facilitate the identification of potential therapeutic targets.

We conducted the present Shizuoka Lung Cancer Mutation Study to assess genomic aberrations in patients with thoracic malignancies. A biobank system was established in collaboration with a clinic pathology lab in July 2011. Mutational data were communicated to clinicians and utilized for assigning patients to appropriate therapy and/or enrolling them in clinical trials. Here we report the genomic aberrations identified in patients with SCLC in the Shizuoka study.

2. Materials and methods

2.1. Patients

We collected samples of SCLC from a biobank system and analyzed these to determine their molecular profiles. To evaluate the relationships between any genomic aberrations and patient characteristics, we collected patient demographic and clinical data from medical records. All patients who participated in this study provided their written informed consent.

Pathological diagnoses were made by institutional pathologists according to the 2004 World Health Organization classification based on morphology (uniform round to spindle-shaped small cells, sparse cytoplasm, high mitotic index, and necrotic areas). The diagnosis of SCLC was confirmed when necessary by immunohistochemical analyses of neuroendocrine markers (synaptophysin, chromogranin A, and CD56). And when it is difficult to diagnose samples as SCLC, we additionally performed immunohistochemistry with makers, such as CAM5.2, TTF-1 and Keratin. If more than 10% of a sample comprised adenocarcinoma, the patient was diagnosed with combined SCLC and adenocarcinoma. Surgically resected samples were macrodissected before nucleic acid extraction and tumor biopsy samples with 10% or more tumor cell component were tested for mutational profiling [12]. All of pleural effusion samples were confirmed that malignant cells were present in each pleural effusion by cytology and we analyzed the cytologically confirmed pleural effusion specimens subsequently.

Smokers were defined according to the Brinkman index (BI) as light (BI value < 600) or heavy (BI value ≥ 600) smokers. Limited stage-disease was defined as disease confined to one hemithorax, the ipsilateral supraclavicular fossa, or both. Disease not meeting these criteria was defined as extended-stage disease. Serum neuron-specific enolase (NSE) levels were measured using a solid-phase radioimmunoassay (RIA) method (SRL Inc., Tokyo, Japan), and progastrin-releasing peptide (Pro-GRP) levels were measured using an enzyme-linked immunosorbent assay (ELISA) kit (FUJIREBIO Inc., Tokyo, Japan).

2.2. Clinical genotyping

We developed a multiplexed tumor genotyping platform to assess 23 mutations in nine genes (*EGFR*, *KRAS*, *BRAF*, *PIK3CA*, *NRAS*, *MEK1*, *AKT1*, *PTEN*, and *HER2*), *EGFR*, *MET*, *PIK3CA*, *FGFR1*, and *FGFR2* amplifications, and *EML4-ALK*, *KIF5B-RET*, *CD74-ROS1*, and *SLC34A2-ROS1* fusion genes (Table 1).

Table 1
Multiplexed tumor genotyping panel.

Gene name	Position	AA mutant	Nucleotide mutant
EGFR	G719	G719	2155G>T/A
		G719A	2156G>C
	exon 19	Deletion	
	T790	T790M	2369C>T
	exon 20	Insertion	
	L858	L858R	2573T>G
	L861	L861Q	2582T>A
KRAS	G12	G12C/S/R	34G>T/A/C
		G12V/A/D	35G>T/C/A
	G13	G13C/S/R	37G>T/A/C
		G13D/A	38G>A/C
	Q61	Q61K	181C>A
		Q61R/L	182A>G/T
		Q61H	183A>T/C
BRAF	G466	G466V	1397G>T
	G469	G469A	1406G>C
	L597	L597V	1789C>G
	V600	V600E	1799T>A
PIK3CA	E542	E542K	1624G>A
	E545	E545K/Q	1633G>A/C
	H1047	H1047R	3140A>G
NRAS	Q61	Q61K	181C>A
		Q61L/R	182A>T/G
MEK1 (MAP2K1)	Q56	Q56P	167A>C
	K57	K57N	171G>T
	D67	D67N	199G>A
AKT1	E17	E17K	49G>A
PTEN	R233	R233*	697C>T
HER2	exon 20	Insertion	

2.3. Nucleic acid sample preparation

DNA samples were extracted from surgically resected tissues, body cavity fluids, and tumor biopsy sections using a QIAamp DNA mini kit (QIAGEN, Hilden, Germany) or a QIAamp DNA formalin-fixed paraffin-embedded (FFPE) tissue kit (QIAGEN). The DNA concentration was measured using a Quant-iT PicoGreen dsDNA assay kit (Invitrogen, Carlsbad, CA). Total RNAs were isolated with an RNeasy Mini kit (QIAGEN) and measured using a spectrophotometer (NanoDrop 2000C; Thermo Scientific, Wilmington, DE).

2.4. Pyrosequencing

Pyrosequencing was used to detect single base substitution-type mutations. An internal fragment of each gene was amplified by polymerase chain reaction (PCR) using primers specific for each gene and a PyroMark PCR kit (QIAGEN). The resulting PCR products were sequenced with the PyroMark Q24 (QIAGEN) pyrosequencer using PyroMark Gold Q96 reagents (QIAGEN) and sequencing primers specific for each gene.

2.5. Fragment analysis

Insertion/deletion-type mutations were identified by sizing the PCR-amplified products using capillary electrophoresis (QIAxcel, QIAGEN).

2.6. Gene copy number analysis

Copy number was evaluated by quantitative real-time PCR (qRT-PCR) performed on a StepOnePlus Real time PCR system (Applied

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Biosystems) using SYBR® Premix Ex Taq™ II (Tli RNaseH Plus) (TAKARA BIO) and PCR primers for each gene. If the gene copy number from the samples was more than double that of the cell line known to be normal human genomic DNA, it was considered as evidence of amplification. Detailed methods are described previously [12].

2.7. Screening for transcripts of fusion genes

Fusion genes were detected by multiplex RT-PCR. Synthesis of cDNA templates was performed with total RNA (1 μg) using Oligo $(dT)_{12-18}$ Primer (Invitrogen) and Omniscript RT (QIAGEN) kits. *EML4-ALK* and *ROS1* fusion genes were detected according to the methods of Sun et al. [13] and Li et al. [14], respectively. Methods for the detection of *KIF5B-RET* fusions were kindly provided by Dr. Takashi Kohno (National Cancer Center, Tokyo).

2.8. Statistical analysis

All categorical variables were analyzed by the chi-square test or Fisher's exact test, as appropriate. Continuous variables, including tumor markers, were analyzed using the Mann–Whitney test. All *p*-values were reported to be two-sided, and values of <0.05 were considered statistically significant. All statistical analyses were performed using JMP version 9.0 software (SAS Institute Inc., Cary, NC, USA). Our study was approved by the Institutional Review Board.

3. Results

3.1. Patient characteristics

Between July 2011 and January 2013, SCLC samples from 60 patients were assessed for genomic aberrations. The patient characteristics are shown in Table 2. The median age (range) was 69 (43–82) years, and most patients were male (83%) and heavy smokers (80%). Only two patients were never-smokers. A total of 57 patients were diagnosed with SCLC, while three were diagnosed with combined SCLC and adenocarcinoma. Thirty-one patients had limited-stage disease and 29 had extended-stage disease. We analyzed eight surgically resected snap-frozen samples, 50 FFPE samples, and seven pleural effusion samples. Five patients provided two specimens: three provided both FFPE and surgically resected snap-frozen samples and two provided both FFPE and pleural effusion samples (Table 3).

Table 2
Patients characteristics that were analyzed in our study (overall, *N* = 60).

	N = 60	%
Median age (years)	69	
Range	43–82	
Gender		
Male	50	83
Female	10	17
Smoking status		
Never	2	3
Light (B.I. < 600)	10	17
Heavy (B.I. ≥ 600)	48	80
Histology		
Small cell carcinoma	57	95
Combined small cell carcinoma with adenocarcinoma	3	5
Disease extent		
Limited stage	31	52
Extended stage	29	48
Samples		
Surgically resected snap-frozen samples	8	
FFPE samples	50	
Pleural effusion	7	

Abbreviation: B.I., Brinkman index; FFPE, Formalin-fixed paraffin-embedded.

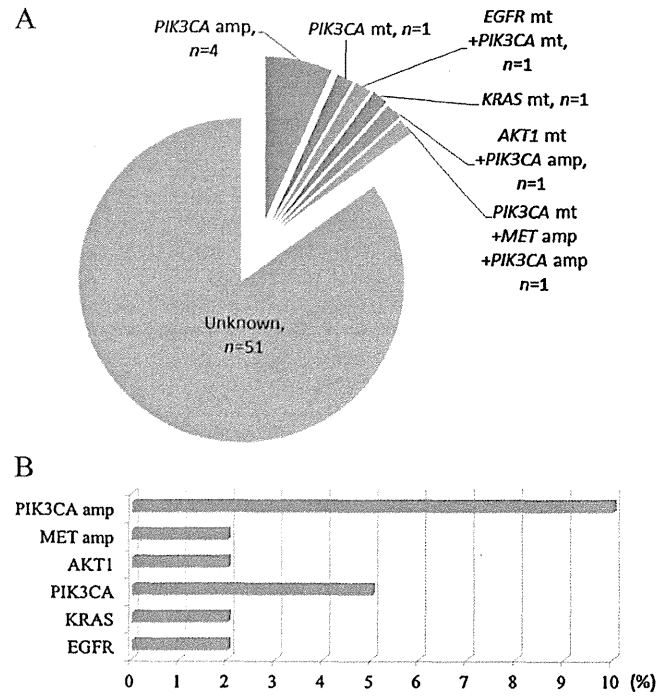

Fig. 1. Relative proportions of genomic aberrations in small cell lung cancer (*N* = 60). (A) Pie chart shows relative proportions of genomic aberrations. (B) Bar chart shows relative proportions of genomic aberrations. *Abbreviations*: mt: mutation; amp: amplification.

3.2. Genomic aberrations

We detected 13 genomic aberrations in nine cases (15%): an *EGFR* mutation (*n* = 1, G719A), a *KRAS* mutation (*n* = 1, G12D), *PIK3CA* mutations (*n* = 3; E542K, E545K, E545Q), an *AKT1* mutation (*n* = 1, E17K), a *MET* amplification (*n* = 1), and *PIK3CA* amplifications (*n* = 6; Fig. 1A and B).

Table 4 shows the individual characteristics of the SCLC patients who harbored genomic aberrations. Eight of the nine patients with genomic aberrations were male, and all were smokers. Two patients were diagnosed with SCLC combined with adenocarcinoma; an *EGFR* mutation was detected in one patient and a *KRAS* mutation in another. The patient with the *EGFR* mutation provided both FFPE and surgically resected snap-frozen samples, but the *EGFR* mutation was detected only in the snap-frozen samples. Genomic aberrations were detected in nine of the 50 FFPE samples, one of eight surgically resected snap-frozen samples, and none of the seven pleural effusion samples.

3.3. Comparison of patient characteristics and genomic aberrations

Patient characteristics are classified by genomic aberration status in Table 4. No significant differences in age, sex, disease extent at diagnosis, or smoking status were found between patients with and without genomic aberrations according to univariate analysis. However, serum NSE and Pro-GRP levels at diagnosis were significantly higher in patients without genomic aberrations than in those with genomic aberrations ($p = 0.02$ and $p = 0.04$, respectively).

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Table 3
Patients characteristics that genomic aberrations were detected.

	Age	Gender	B.I.	Disease extent	TNM stage	Samples	Pathology	Genomic aberrations
1	73	Male	2760	LS	IA	FFPE	Small cell carcinoma	PIK3CA amp (3.14)
2	69	Male	1880	LS	IIA	FFPE	Small cell carcinoma	PIK3CA amp (4.42)
3	82	Male	1500	LS	IIIA	FFPE	Small cell carcinoma	PIK3CA amp (2.65)
4	58	Male	1000	ES	IV	FFPE	Small cell carcinoma	PIK3CA (E545K)
5	69	Male	940	LS	IIIA	FFPE	Small cell carcinoma	AKT1 (E17K), PIK3CA amp (2.49)
6	66	Male	840	ES	IIIB	FFPE	Small cell carcinoma	PIK3CA (E542K), MET amp (4.13), PIK3CA amp (3.62)
7	73	Male	795	LS	IIB	FFPE, snap-frozen samples	Small cell carcinoma combined with adenocarcinoma	EGFR (G719A), PIK3CA (E545Q)
8	74	Male	590	ES	IV	FFPE	Small cell carcinoma combined with adenocarcinoma	KRAS (G12D)
9	80	Female	500	LS	IIA	FFPE	Small cell carcinoma	PIK3CA amp (2.78)

Abbreviations: LS, limited stage; ES, extended stage; FFPE, formalin-fixed paraffin-embedded.

Table 4
Patients characteristics classified by genomic aberration status.

	Genomic aberration		*P* value
	Detected	Not detected	
N (%)	9 (15%)	51 (85%)	
Age at diagnosis (years)			0.26
Median	73	69	
Range	58–82	43–82	
Gender, *n* (%)			0.63
Male	8 (89%)	42 (82%)	
Female	1 (11%)	9 (18%)	
Disease extent at diagnosis, *n* (%)			0.32
Limited stage	6 (67%)	25 (49%)	
Extended stage	3 (33%)	26 (51%)	
Smoking status			0.78
Never	0	2	
Light (B.I. < 600)	2	8	
Heavy (B.I. ≥ 600)	7	41	
Serum neuron-specific enolase (NSE) level at diagnosis			0.02
n	9	48	
Median	14	37.1	
Range	7.8–34	6.4–334	
Serum pro-gastrin releasing peptide (Pro-GRP) level at diagnosis			0.04
n	8	47	
Median	75.5	738	
Range	43.1–1500	26.4–65900	

Abbreviation: B.I., Brinkman index.

4. Discussion

As per our knowledge, this was the first molecular profiling report of Asian patients with SCLC, wherein we detected genomic aberrations in 15% patients. *PIK3CA* amplifications were detected in 10% of all samples assessed, while *PIK3CA* mutations were detected in 5%. *PIK3CA* genomic aberrations were detected in eight of the nine patients with genomic aberrations. Recently, two independent comprehensive genomic studies of SCLC were published [15,16]. Peifer et al. [14] analyzed 99 SCLC specimens using 6.0 SNP array analyses and exome, transcriptome, and genome sequencing. They detected *TP53* and *RB1* alterations in 88% and 66% cases, respectively, *MYC* family member and *FGFR1* amplifications in 16% and 6% cases, respectively, and *CREBBP* and *EP300* and *PTEN* mutations in 18% and 10% cases, respectively. They did not detect any *PIK3CA* aberrations. Rudin et al. [15] analyzed 80 SCLC samples, including SCLC cell lines, using multiple exome sequencing, single genome analysis, genome-wide copy-number analysis, and whole-transcriptome sequencing and detected *TP53* and *RB1* mutations in 77% and 31% samples, respectively, a *SOX2* amplification in 27%, and a recurrent *RLF-MYCL1* fusion in 9%. In their study, *PIK3CA* mutation was detected in 2 of 30 primary SCLC tumor samples by exome capture followed by next generation sequencing (Rudin's report online methods). Recently, Umemura et al. undertook a comprehensive genomic analysis of SCLC in Japanese patients [17]. They analyzed 51 surgically resected SCLC samples using whole exome sequencing and copy-number analysis. Genetic alterations in the *PI3K* pathway (*PIK3CA, PTEN, AKT2, AKT3, RICTOR, mTOR*) were detected in 17 of 47 samples (36%). *PIK3CA* mutations were detected in three of the 47 samples (6%), which is consistent with the findings from our study.

Okudela et al. reported that *PIK3CA* amplification was detected in 1 of 3 samples (33.3%) and *PIK3CA* gene mutation was detected in

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1 of 5 samples (20%) in Japanese patients with SCLC [18]. Although *PIK3CA* mutation is the major genomic aberration in Japanese SCLC patients, the larger study, such as our study and Umemura's report, detected it in approximately 5% of SCLC samples. Based on these results, there does not seem to be significant ethnic differences in the prevalence of *PIK3CA* mutation and *PIK3CA* mutation may be one of the major genomic alterations for the SCLC patients. The *PI3K* pathway plays a central role in cell proliferation and survival in human cancer [19]. The *PIK3CA* gene encodes a class IA PI3K catalytic subunit p110α and is frequently mutated in some of the most common human tumors [20]. Wojtalla et al. showed that approximately 25% primary SCLC tissue samples overexpress the PI3K isoform p110α [21]. They also reported that targeting PI3K p110α affected the proliferation of SCLC cells *in vitro* and *in vivo* and that p110α inhibition led to impaired SCLC tumor formation and vascularization *in vivo*. Many drugs targeting class IA PI3K have been developed [22], and preclinical studies have shown these to have potent antitumor activity. Some have led to a decrease in advanced solid tumors in phase I studies [23,24]; therefore, *PIK3CA* may be a suitable target for the treatment of SCLC.

EGFR and *KRAS* mutations were detected in the patients with combined SCLC and adenocarcinoma in our study. Tatematsu et al. analyzed 122 SCLC patients and detected *EGFR* mutations in 5 (4%) [25]. Their study included 15 combined subtype patients, and 20% of these had *EGFR* mutations. Compared with conventional SCLC, *EGFR* mutations are found significantly more frequently in the combined subtype. Fukui et al. retrospectively studied six patients with combined SCLC and adenocarcinoma and analyzed the *EGFR* mutation status in the microdissected SCLC and adenocarcinoma components of their resected samples [26]. In their report, one of six patients had a missense mutation in *EGFR* (L858R), and both the SCLC and adenocarcinoma components shared the same mutation. Gene mutation status in tissue samples from SCLC with other histology component remain an open question. Therefore it is necessary to perform microdissection in the future study. To the best of our knowledge, there has been no previous report of *KRAS* mutations in SCLC. In our study, a *KRAS* mutation was detected in one patient with combined SCLC and adenocarcinoma.

No significantly different characteristics were found between patients with and without genomic aberrations in the present study. Although the associations between serum tumor markers and genomic aberrations were unclear, serum NSE and pro-GRP levels at diagnosis were significantly lower in the patients with genomic aberrations. Pujol et al. reported that pro-GRP levels did not have any independent prognostic significance [27], while NSE levels have been shown to have better prognostic value [28]. We could not detect an association between prognosis and genomic aberration status (data not shown). Further studies are needed to clarify the relationships between genomic aberrations and serum tumor marker values.

In this study, genomic aberrations were detected in 18% FFPE samples and 13% surgically resected snap-frozen samples. The National Comprehensive Cancer Network (NCCN) guideline recommends that surgery should only be considered for patients with stage I SCLC. However, another report stated that only 5% patients with SCLC have true stage I SCLC [29]. Because surgery is not performed in most patients with SCLC, FFPE samples play a key role in detecting genomic aberrations. Kenmotsu et al. reported on the concordance between FFPE samples and surgically resected snap-frozen samples in multiplexed molecular profiling of lung cancers [30]. Complete concordance of driver mutations was shown for 65% FFPE and snap-frozen samples. These findings indicate that it may be better to analyze FFPE samples to identify SCLC molecular profiles and treat patients with molecular-targeted drugs such as PI3K inhibitors.

Our study had several limitations. First, we analyzed SCLC genomic aberrations using a nine-gene tumor genotyping panel, not a comprehensive panel. In addition, we did not include some known driver mutations such as *TP53* and *RB1* mutations in the panel. However, the objectives of our study were not only to assess the frequency of genomic aberrations but also to detect genomic aberrations that are treatable with targeted drugs, and our multiplexed tumor genotyping platform includes almost all known gene aberrations that are targeted by drugs. And detection of gene amplification may also require consideration of incorporating FISH for future studies. Second, we only analyzed 60 SCLC patients because we only began to analyze genomic aberrations in July 2011. However, other reports have also included a small number of samples. We continue to analyze SCLC samples and utilize the findings for targeted therapy of patients with SCLC.

5. Conclusions

In conclusion, genomic aberrations were found in 15% SCLC patients, with *PIK3CA* amplifications being frequently detected. We previously reported our massive parallel sequencing findings for non-SCLC [31], and we plan to undertake a similar analysis of SCLC samples. A larger study is necessary to further our understanding of the molecular profiles of SCLC.

Conflicts of interest

None of the authors have any financial or personal relationship with other individuals or organizations that could inappropriately influence this study.

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References

[1] van Meerbeeck JP, Fennell DA, De Ruysscher DK. Small-cell lung cancer. Lancet 2011;378(9804):1741–55.
[2] Puglisi M, Dolly S, Faria A, Myerson JS, Popat S, O'Brien ME. Treatment options for small cell lung cancer – do we have more choice? Br J Cancer 2010;102(4):629–38.
[3] Lynch TJ, Bell DW, Sordella R, Gurubhagavatula S, Okimoto RA, Brannigan BW, et al. Activating mutations in the epidermal growth factor receptor underlying responsiveness of non-small-cell lung cancer to gefitinib. N Engl J Med 2004;350(21):2129–39.
[4] Paez JG, Janne PA, Lee JC, Tracy S, Greulich H, Gabriel S, et al. EGFR mutations in lung cancer: correlation with clinical response to gefitinib therapy. Science 2004;304(5676):1497–500.
[5] Mitsudomi T, Morita S, Yatabe Y, Negoro S, Okamoto I, Tsurutani J, et al. Gefitinib versus cisplatin plus docetaxel in patients with non-small-cell lung cancer

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harbouring mutations of the epidermal growth factor receptor (WJTOG3405): an open label, randomised phase 3 trial. Lancet Oncol 2010;11(2):121–8.
[6] Maemondo M, Inoue A, Kobayashi K, Negoro S, Okamoto I, Tsurutani J, et al. Gefitinib or chemotherapy for non-small-cell lung cancer with mutated EGFR. N Engl J Med 2010;362(25):2380–8.
[7] Zhou C, Wu YL, Chen G, Feng J, Liu XQ, Wang C, et al. Erlotinib versus chemotherapy as first-line treatment for patients with advanced EGFR mutation-positive non-small-cell lung cancer (OPTIMAL CTONG-0802): a multicentre, open-label, randomised, phase 3 study. Lancet Oncol 2011;12(8):735–42.
[8] Rosell R, Carcereny E, Gervais R, Vergnenegre A, Massuti B, Felip E, et al. Erlotinib versus standard chemotherapy as first-line treatment for European patients with advanced EGFR mutation-positive non-small-cell lung cancer (EURTAC): a multicentre, open-label, randomised phase 3 trial. Lancet Oncol 2012;13(3):239–46.
[9] Ettinger DS, Akerley W, Borghaei H, Chang AC, Cheney RT, Chirieac LR, et al. Non-small cell lung cancer. J Natl Compr Cancer Netw 2012;10(10):1236–71.
[10] Peters S, Adjei AA, Gridelli C, Reck M, Kerr K, Felip E. Metastatic non-small-cell lung cancer (NSCLC): ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. Ann Oncol 2012;23(Suppl. 7):vii56–64.
[11] Planchard D, Le Pechoux C. Small cell lung cancer: new clinical recommendations and current status of biomarker assessment. Eur J Cancer 2011;47(Suppl. 3):S272–83.
[12] Serizawa M, Koh Y, Kenmotsu H, Isaka M, Murakami H, Akamatsu H, et al. Assessment of mutational profile of Japanese lung adenocarcinoma patients by multitarget assays: a prospective single-institute study cancer; 2014 (in press).
[13] Sun Y, Ren Y, Fang Z, Li C, Fang R, Gao B, et al. Lung adenocarcinoma from East Asian never-smokers is a disease largely defined by targetable oncogenic mutant kinases. J Clin Oncol 2010;28(30):4616–20.
[14] Li C, Fang R, Sun Y, Han X, Li F, Gao B, et al. Spectrum of oncogenic driver mutations in lung adenocarcinomas from East Asian never smokers. PLoS ONE 2011;6(11):e28204.
[15] Peifer M, Fernandez-Cuesta L, Sos ML, George J, Seidel D, Kasper LH, et al. Integrative genome analyses identify key somatic driver mutations of small-cell lung cancer. Nat Genet 2012;44(10):1104–10.
[16] Rudin CM, Durinck S, Stawiski EW, Poirier JT, Modrusan Z, Shames DS, et al. Comprehensive genomic analysis identifies SOX2 as a frequently amplified gene in small-cell lung cancer. Nat Genet 2012;44(10):1111–6.
[17] Umemura S, Goto K, Mimaki S, Ishii G, Ohmatsu H, Niho S, et al. Comprehensive genomic analysis of small cell lung cancer in Asian patients. ASCO Meet Abstr 2013;31(Suppl. 15):7512.
[18] Okudela K, Suzuki M, Kageyama S, Bunai T, Nagura K, Igarashi H, et al. PIK3CA mutation and amplification in human lung cancer. Pathol Int 2007;57(10):664–71.
[19] Luo J, Manning BD, Cantley LC. Targeting the PI3K-Akt pathway in human cancer: rationale and promise. Cancer Cell 2003;4(4):257–62.
[20] Samuels Y, Wang Z, Bardelli A, Silliman N, Ptak J, Szabo S, et al. High frequency of mutations of the PIK3CA gene in human cancers. Science 2004;304(5670):554.
[21] Wojtalla A, Fischer B, Kotelevets N, Mauri FA, Sobek J, Rehrauer H, et al. Targeting the phosphoinositide 3-kinase p110-alpha isoform impairs cell proliferation, survival, and tumor growth in small cell lung cancer. Clin Cancer Res 2013;19(1):96–105.
[22] Liu P, Cheng H, Roberts TM, Zhao JJ. Targeting the phosphoinositide 3-kinase pathway in cancer. Nat Rev Drug Discov 2009;8(8):627–44.
[23] Gonzalez-Angulo AM, Juric D, Argiles G, Schellens JH, Burris HA, Berlin J, et al. Safety, pharmacokinetics, and preliminary activity of the α-specific PI3K inhibitor BYL719: results from the first-in-human study. ASCO Meet Abstr 2013;31(Suppl. 15):2531.
[24] Omlin AG, Spicer JF, Sarker D, Pinato DJ, Agarwal R, Cassier PA, et al. A pharmacokinetic (PK) pharmacodynamic (PD) driven first-in-human study of the oral class I PI3K inhibitor CH5132799, in patients with advanced solid tumors. ASCO Meet Abstr 2012;30(Suppl. 15):3022.
[25] Tatematsu A, Shimizu J, Murakami Y, Horio Y, Nakamura S, Hida T, et al. Epidermal growth factor receptor mutations in small cell lung cancer. Clin Cancer Res 2008;14(19):6092–6.
[26] Fukui T, Tsuta K, Furuta K, Watanabe S, Asamura H, Ohe Y, et al. Epidermal growth factor receptor mutation status and clinicopathological features of combined small cell carcinoma with adenocarcinoma of the lung. Cancer Sci 2007;98(11):1714–9.
[27] Pujol JL, Quantin X, Jacot W, Boher JM, Grenier J, Lamy PJ. Neuroendocrine and cytokeratin serum markers as prognostic determinants of small cell lung cancer. Lung Cancer 2003;39(2):131–8.
[28] Jorgensen LG, Osterlind K, Genolla J, Gomm SA, Hernandez JR, Johnson PW, et al. Serum neuron-specific enolase (S-NSE) and the prognosis in small-cell lung cancer (SCLC): a combined multivariable analysis on data from nine centres. Br J Cancer 1996;74(3):463–7.
[29] Ignatius Ou SH, Zell JA. The applicability of the proposed IASLC staging revisions to small cell lung cancer (SCLC) with comparison to the current UICC 6th TNM Edition. J Thorac Oncol 2009;4(3):300–10.
[30] Kenmotsu H, Serizawa M, Koh Y, Isaka M, Takahashi T, Murakami H, et al. Concordance between formalin-fixed paraffin-embedded biopsy samples and surgically resected snap-frozen samples in multiplexed molecular profiling of lung cancers. ASCO Meet Abstr 2013;31(Suppl. 15):e18556.
[31] Koh Y, Kenmotsu H, Serizawa M, Isaka M, Mori K, Imai H, et al. Identification of actionable mutations in surgically resected tumor specimens from Japanese patients with non-small cell lung cancer by ultra-deep targeted sequencing. ASCO Meet Abstr 2013;31(Suppl. 15):7572.

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Clinical Trial Notes

A Phase III Trial Comparing Irinotecan and Cisplatin with Etoposide and Cisplatin in Adjuvant Chemotherapy for Completely Resected Pulmonary High-grade Neuroendocrine Carcinoma (JCOG1205/1206)

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A randomized Phase III trial commenced in Japan in March 2013. Post-operative adjuvant chemotherapy with etoposide plus cisplatin is the current standard treatment for resected pulmonary high-grade neuroendocrine carcinoma including small cell lung cancer and large cell neuroendocrine carcinoma. The purpose of this study is to confirm the superiority of irinotecan plus cisplatin in terms of overall survival over etoposide plus cisplatin as post-operative adjuvant chemotherapy for pathological Stage I–IIIA completely resected pulmonary high-grade neuroendocrine carcinoma patients. A total of 220 patients will be accrued from 54 Japanese institutions within 6 years. The primary endpoint is overall survival and the secondary endpoints are relapse-free survival, proportion of treatment completion, adverse events, serious adverse events and second malignancy. This trial has been registered at the UMIN Clinical Trials Registry as UMIN000010298 [http://www.umin.ac.jp/ctr/index.htm].

Key words: lung neoplasms – high-grade neuroendocrine carcinoma – adjuvant chemotherapy – Phase III

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INTRODUCTION

Lung cancer has been the leading cause of cancer-related deaths in Japan since 1988. High-grade neuroendocrine carcinoma (HGNEC) including small cell lung cancer (SCLC) and large cell neuroendocrine carcinoma (LCNEC) accounts for ~15% of all lung cancers (1,2).

LCNEC was first proposed by Travis et al. (3), who added LCNEC as the fourth category of pulmonary neuroendocrine tumors, which had originally been classified into three categories, typical carcinoid, atypical carcinoid and SCLC. Although it has been classified into a non-small cell lung cancer (NSCLC) by the WHO classification, LCNEC has neuroendocrine features and an aggressive clinical course that are common with SCLC and both are recognized as HGNEC. LCNEC is typically diagnosed post-operatively using surgical specimens and rarely diagnosed pre-operatively with biopsy specimens because of the difficulties associated with its diagnosis from a small amount of specimens. Furthermore, a differential diagnosis between LCNEC

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