between elderly and younger patients with NSCLCs. Given that smoking is one of the causes of the low rate of *EGFR* mutations in the older group, the rate of *EGFR* mutations may increase in the future owing to enlightenment movements such as the WHO Framework Convention on Tobacco Control [19]. Recently, smoking prevalence in Japan is decreasing generally. In particular, the drop of the smoking prevalence in young generation is remarkable. On the other hands, lung cancer mortality in Japan rises, probably it depends on the increase of the lung cancer in an elderly person who had been a smoker [20]. If the low rate of *EGFR* mutations is unrelated to smoking, it is very interesting that EGFR status might be affected by aging. Furthermore, it is reported that the response rate of gefitinib in elderly (aged 70 years or older) patients with advanced *EGFR* mutated NSCLC was 45.5%. EGFR-TKI is more effective than conventional chemotherapy in elderly patients, if we could pay attention to drug discontinuation and dose reduction due to age-related organ dysfunction [21]. On the other hand, NSCLC with exon 20 mutation is resistant for EGFR-TKI. Although our result has no statistical significance due to a small population of elderly patients, the lack of exon 20 mutations might be a characteristic of elderly patients. Large clinical trials are needed to investigate the relation between age group and the response to EGFR-TKI.

Finally, we assessed the relations between the *EGFR* status and outcomes. *EGFR* mutations were associated with significantly better survival than wild-type *EGFR* in the younger group (Figure 1). In the older group, however, the 5-year overall survival rate did not differ significantly according to *EGFR* mutations, and wild-type *EGFR* status and was 100% in patients with *EGFR* mutations. EGFR-TKIs are obviously beneficial in patients with advanced or recurrent NSCLC, but several studies have suggested that *EGFR* mutations might be an independent positive prognostic factor [22]. Our results suggest that elderly patients with NSCLC who have *EGFR* mutations are especially likely to have good outcomes after complete lung resection.

Conclusion

Our results suggest that the *EGFR* status of patients with NSCLC differs according to age group (>80 years vs. ≤80 years). *EGFR* mutation status might be a prognostic marker in elderly patients with completely resected NSCLC.

Additional file

Additional file 1: Table S1. PCR Primers and LH-G Probes Used for Detection of Mutations in *EGFR*.

Competing interests

The authors declare that they have no competing interest.

Authors' contributions

Study design: TN, TY, YM and YD; sample collection: TN, HI, TI, KI, Shuji M, TK, HS, FO, KY, MT, and HN; experiments: TN, TY, YM, YD, and Shoichi M; data analysis: TN and TY; preparation of the manuscript: TN, TY, HN, and MM. All authors read and approved the final manuscript.

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Author details

[1]Department of Thoracic Oncology, Kanagawa Cancer Center Hospital, 2-3-2 Nakao, Asahi-ku, Yokohama 2418515, Japan. [2]Department of Pathology, Kanagawa Cancer Center Hospital, 2-3-2 Nakao, Asahi-ku, Yokohama 2418515, Japan. [3]Molecular Pathology and Genetics Division, Kanagawa Cancer Center Research Institute, 2-3-2 Nakao, Asahi-ku, Yokohama 2418515, Japan. [4]Department of Medical Oncology and Cancer Center, Shiga University of Medical Science Hospital, Seta Tsukinowa-cho, Otsu 5202192, Japan. [5]Respiratory Disease Center, Yokohama City University Medical Center, 4-57 Urafune-cho, Minami-ku, Yokohama 2320024, Japan. [6]Department of Surgery, Yokohama City University Graduate School of Medicine, 3-9 Fukuura, Kanazawa-ku, Yokohama 2360004, Japan.

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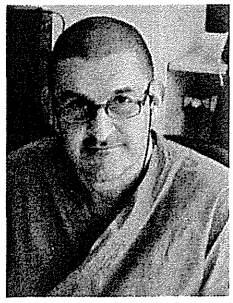
Preface

Knowledge of Pulmonary Neuroendocrine Tumors: Where Are We Now?



Pier Luigi Filosso, MD, FECTS, FCCP
Editor

Neuroendocrine tumors (NETs) of the lung are regarded as a distinct clinical subgroup of lung cancer, which share particular morphologic, ultrastructural, immunohistochemical, and molecular characteristics. According to the 2004 World Health Organization classification of tumors,[1] they are categorized into 4 major groups,[2] ranging from the low-grade typical carcinoid (TC), to highly aggressive, poorly differentiated tumors (large-cell neuroendocrine carcinoma, LCNC, and small-cell lung cancer, SCLC). Amid them, an intermediate-grade neoplasm (atypical carcinoid, AC) is characterized by a greater aggressive biological behavior, compared to TC with a poorer 5-year survival and a higher tendency to lymph-nodal involvement at presentation. TCs and ACs are categorized together as carcinoids; LCNC is considered a subgroup of large-cell carcinomas, and SCLC is an independent class of lung cancer.

NETs derive from the pulmonary neuroendocrine cells (PNECs), which are of endodermal origin, regardless of their phenotypic resemblance to neurons.[3] In the postnatal phase and later, the PNEC system represents the lung stem cells niche, which is extremely important in the airway epithelial regeneration and carcinogenesis.[4,5] In the healthy adult, the PNECs distribution is quite permeating, with approximately 1 PNEC for every 2500 epithelial cells. Although PNECs are mostly solitary, sometimes they appear aggregate in innervated PNEC clusters, intended as neuroepithelial bodies (NEBs).[6] The precise PNEC biological function remains unclear, as well as that of NEBs. Singular PNEC and NEB have a similar phenotype, because they are the site of adenosine, serotonin, and other amines storage, which play a very important role in normal lung development, growth, and repair. They have been considered to serve as airway chemoreceptors, responsive to hypoxia and thought to activate vagal nerves, participating in breath regulation.[7]

Neuroendocrine cell spread is also thought to be a rare preneoplastic condition: diffuse idiopathic pulmonary neuroendocrine cell hyperplasia (DIPNECH) is, in fact, characterized by a widespread peripheral airway PNEC and NEBs proliferation, while Tumorlet is a nodular neuroendocrine cell proliferation that measures less than 5 mm in diameter. DIPNECHs are also considered a sort of adaptive response in persons that live at high altitudes, as well as a reactive response during lung injuries, the commonest of which are obliterative bronchiolitis and interstitial lung disease, and in patients with chronic cough.[8–10]

Genetic abnormalities have been recently detected and proposed for a better classification of lung NETs. In particular, abnormal expression or loss of heterozygosity and point mutations of the p53 locus on chromosome 17p13 were seen in approximately 4% of TCs, 29% of ACs, and 80%

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of LCNCs; this data may support the hypothesis that TC, AC, and LCNC are genetically different from each other.[11,12] Also, the p53 protein frequency was found to be 0% in TCs, 20% in ACs, and 80% in LCNCs, suggesting that this data could be used to better classify these neoplasms.[13]

The recent improvement in histologic diagnostic tools, as well as the rapid diffusion of lung cancer screening programs, resulted in a recent increase in pulmonary NETs recognition; this may explain their rapid growth in incidence, which actually accounts for approximately 30% of all NETs.[14]

Lung NETs comprise roughly 20% of all primary lung cancers; their incidence has been reported to be 1.57/100,000/year, with a median age at presentation of 64 years.

Bronchial carcinoids (both TCs and ACs) have an annual incidence comprised between 2.3 and 2.8 cases/1,000,000 people[15] and include 20% to 25% of all carcinoid tumors, but account for only 3% of all primary lung cancers. Bronchial carcinoids have an equal gender distribution; in the retrospective series, the median age of patients with TC is lower than for those diagnosed with ACs or other neuroendocrine neoplasms.

The majority of TCs are centrally located; whereas ACs and LCNCs tend to be more frequently peripheral, ACs sometimes are greater in size. Despite that patients diagnosed with SCLC and LCNC are likely to have a heavy smoking history, a clear correlation between tobacco exposure and carcinoid development has not yet been demonstrated, even if Fink and colleagues[15] and Filosso and coworkers[16] observed a higher frequency of smokers in their AC group.

Peripheral lesions tend to be asymptomatic, whereas cough, dyspnea, pneumonitis, and hemoptysis are the commonest symptoms in centrally located lesions; in addition, symptoms may be present for many years before the diagnosis, reflecting a possible slow tumor growth.

Paraneoplastic syndromes occur in less than 5% of NETs and are more frequently associated with bronchial carcinoids and SCLCs.

Cushing syndrome, due to an ectopic adrenocorticotropic hormone production and secretion, may occur in less than 2% of carcinoids, whereas less than 1% of patients with Cushing syndrome have a bronchopulmonary carcinoid.

Carcinoid syndrome, characterized by symptoms related to serotonin secretion (diarrhea, wheezing, flushing, and carcinoid heart disease), is very rare (<1% to 3% in bronchial carcinoids) and usually reflects the presence of liver metastases.

The syndrome of inappropriate antidiuretic hormone secretion is the commonest paraneoplastic syndrome in SCLC (approximately 5.5% at the time of diagnosis).[17] It is caused by the antidiuretic hormone disproportionate secretion and is characterized by reduced plasma osmolarity, concentrated urine, and euvolemic hyponatremia.

Less frequent paraneoplastic syndromes include acromegaly, hypercalcemia, hypoglycemia, and myasthenia gravis.

Bronchial carcinoids may also occur as a component (less than 5%) of the familial endocrine cancer syndrome called multiple neuroendocrine neoplasia 1 (MEN1),[18] although the majority occur as sporadic cases. MEN1 is an autosomal-dominant disease, associated with the gene locus on 11q13 and characterized by neoplasms of the pituitary gland, pancreas, and parathyroid.

Surgery is the treatment of choice for bronchial carcinoids; complete tumor resection with preservation of as much lung tissue as possible is to be achieved, whenever feasible. The conservative resection, in the case of TC, could be a sleeve resection (in the case of centrally located lesion), or a segmentectomy or lobectomy. Lobectomy/bilobectomy (depending on the tumor size and its location) may be proposed for AC. Systemic lymphadenectomy[19] must be accomplished in all cases, because lymph nodal metastases are evident in about 40% of ACs.[20]

Surgery achieves a 5- and 10-year survival rate higher than 90% for TCs and 70% and 50%, respectively, for ACs.[20] Recurrences occur in 3% to 5% of TCs, and only 15% of deaths are caused by the tumor, while in ACs the majority of deaths are due to recurrences, which occur in about 26% of cases.

The use of several various chemotherapeutic agents (doxorubicin, 5-fluorouracil, dacarbazine, cisplatin, etoposide, streptozocin, and carboplatin) has been proposed for advanced bronchial carcinoids, but it has yielded minimal and generally short-lasting results.[21] More recently, temozolamide and everolimus have been used, with promising results.

Many LCNCs/SCLCs are poor candidates for surgery, mostly due to their local or systemic spread. Lobectomy and lymphadenectomy are the treatments preferred in early-stage LCNCs, and these procedures may improve survival if no lymph nodal metastases are found. Otherwise, the reported outcome is very poor.[22,23] Recurrences and distant metastases occur early, even after a complete resection and also in stage I tumors[24]; surgery alone does not seem the appropriate treatment and should be followed by chemoradiotherapy.

In SCLC patients with a limited disease (T1-T2 N0), surgery with systemic lymphadenectomy, followed by adjuvant chemoradiotherapy, may be proposed as part of their treatment plan. SCLC is usually extremely sensitive to chemotherapy; the combination of etoposide and cisplatin is most widely used, yielding response rates of 60% to 80%.[25] However, tumor recurrences (or distant metastases) are very common in the first 2 years after the induction treatment.

A great deal of research is needed to better understand the treatment of such rare neoplasms. That is the aim of this publication, which collects papers coming from the most experienced international centers in the scientific community of pathologists, thoracic surgeons, and oncologists.

Two years ago, the European Society of Thoracic Surgeons (www.ests.org) launched a new working group on NETs and a retrospective database was immediately designed. Through this, more than 1900 NETs cases have been collected from several European and American institutions. This database actually represents an important source for future studies and scientific projects, but this is not enough: the next step, in fact, will be the development of a new prospective NETs database, with the aim of collecting the shortest possible time of one of the largest NETs clinical series available for the scientific community. Further efforts by pathologists, biologists, and oncologists are needed to expand the biological behavior of such rare neoplasms, to improve knowledge on their recurrence development mechanisms, as well as on their medical and/or biological treatment. I hope that this publication may serve the scientific community to lead to the development of possible uniform guidelines for NETs management.

I would like to address my special thanks to all the coauthors for their enthusiasm to this project and their strong and valuable effort and expertise in the preparation of their articles. Their help has strongly contributed to improving the quality of this issue of *Thoracic Surgery Clinics*.

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Pier Luigi Filosso, MD, FECTS, FCCP
Department of Thoracic Surgery
University of Torino
San Giovanni Battista Hospital
Via Genova, 3
Torino 10126, Italy

The European Society of Thoracic Surgeons (ESTS)

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E-mail address:
pierluigi.filosso@unito.it

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Surgery for Small Cell Lung Cancer

A Retrospective Analysis of 243 Patients from Japanese Lung Cancer Registry in 2004

Hidefumi Takei, MD, Haruhiko Kondo, MD,* Etsuo Miyaoka, PhD,† Hisao Asamura, MD,‡ Ichiro Yoshino, MD,§ Hiroshi Date, MD,‖ Meinoshin Okumura, MD,¶ Hirohito Tada, MD,# Yoshitaka Fujii, MD,** Yoichi Nakanishi, MD,†† Kenji Eguchi, MD,‡‡ Hirotoshi Dosaka-Akita, MD,§§ Hideo Kobayashi, MD,‖‖ Noriyoshi Sawabata, MD,¶ and Kohei Yokoi, MD;¶¶ for the Japanese Joint Committee of Lung Cancer Registry*

Introduction: Indications for surgical resection for small cell lung cancer (SCLC) have been very limited. Because early-stage SCLC is a rare presentation of lung cancer, studies comparing surgical resection among a large number of patients are unlikely to be conducted. This study reports the most recent surgical outcomes of a large number of SCLC patients who underwent surgery in 2004.Methods: In 2010, the Japanese Joint Committee of Lung Cancer Registry performed a nationwide retrospective registry study regarding the prognosis and clinicopathologic profiles of 11,663 patients who underwent resection for primary lung cancer in 2004. Of the 11,663 patients, 243 patients with SCLC (2.1%) were included in this study. The registry data of the patients with SCLC were analyzed, and the clinicopathologic profiles and surgical outcomes of the patients were evaluated.Results: The 5-year survival rate for all cases (n = 243, 213 males, mean age 68.2 years) was 52.6%. The 5-year survival rates by c-stage and p-stage were as follows: IA, 64.3% (n = 132) and 72.3% (n = 93); IB, 45.7% (n = 36) and 61.1% (n = 51); IIA, 50.5% (n =25); and 44.8% (n = 27); IIB, 33.3% (n = 10) and 40.3% (n = 17); IIIA, 30.5% (n = 30) and 23.4% (n = 45); and IV, 0% (n = 7) and 0% (n = 9), respectively. A multivariate analysis showed that the significant prognostic factors were age, gender, c-stage, and surgical curability. A kappa value was moderate conformity between c-stage and p-stage in all cases.Conclusions: Surgical resection in selected patients with early-stage SCLC, especially stage I, had favorable results.

Key Words: Small cell lung cancer, Surgery, Registry

(*J Thorac Oncol.* 2014;9: 1140–1145)

Lung cancer is the leading cause of cancer-related death in the United States and in Japan. Small cell lung cancer (SCLC) represents only 13–20% of all lung cancers.[1] It is distinguished by its rapid growth rate and early dissemination to regional lymph nodes and distant sites. Therefore, SCLC represents less than 5% of cases in large surgical series.[2]

In 1973, the Medical Research Council[3] reported a postoperative survival rate that was as poor as the survival rate for nonsurgical treatment in SCLC patients. In addition, Mountain[4] reported that there was no difference in outcome between resected and non-resected cases in 368 SCLC patients. After those two studies were published, the standard treatment for SCLC became chemotherapy and/or radiation, and surgery was basically contraindicated. In 1983, the Lung Cancer Study Group[5] initiated the only randomized trial of adjuvant surgical resection after induction chemotherapy. This trial failed to show improved survival rates after surgery compared with radiation after neoadjuvant chemotherapy. Thereafter, several authors reported rather favorable surgical results in a relatively small number of patients with early-stage SCLC.[6,7] Shepherd and colleagues[8] reported in 1988 that the postoperative 5-year survival rate was 31% in 77 patients with surgery as the primary treatment for SCLC. In 2005, Japan Clinical Oncology Group reported a 68% 3-year postoperative survival rate in patients with resected clinical stage I SCLC undergoing postoperative adjuvant chemotherapy.[9] Recently, several large cohort studies of surgery for limited disease SCLC have

*Department of General Thoracic Surgery, Kyorin University, Tokyo; †Department of Mathematics, Science University of Tokyo; ‡Division of Thoracic Surgery, National Cancer Center Hospital, Tokyo; §Department of General Thoracic Surgery, Chiba University Graduate School of Medicine, Chiba; ‖Department of Thoracic Surgery, Kyoto University Graduate School of Medicine, Kyoto, ¶Department of General Thoracic Surgery, Osaka University Graduate School of Medicine, #Division of General Thoracic Surgery, Osaka City General Hospital, Osaka; **Department of Oncology, Immunology and Surgery, Nagoya City University Graduate School of Medical Science and Medical School, Nagoya; ††Department of Clinical Medicine, Research Institute for Diseases of the Chest, Faculty of Medical Sciences, Kyushu University, Fukuoka; ‡‡Department of Medical Oncology, Teikyo University School of Medicine, Tokyo; §§Department of Medical Oncology, Hokkaido University Graduate School of Medicine, Sapporo; ‖‖Division of Respiratory Disease, National Defense Medical College, Tokorozawa; and ¶¶Department of Thoracic Surgery, Nagoya University Graduate School of Medicine, Nagoya, Japan.

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Address for correspondence: Hidefumi Takei, MD, Division of General Thoracic Surgery, Kyorin University, 6-20-2, Shinkawa, Mitaka, Tokyo, 181–8611, Japan. E-mail: htakei@ks.kyorin-u.ac.jp

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been reported.[10,11] An analysis of 205 clinical stage IA SCLC patients from the National Cancer Institute's Surveillance Epidemiology and End Results database who underwent radical lobectomy showed a 5-year survival rate of 50.3% without postoperative adjuvant radiotherapy.[12]

However, optimal indications for surgical resection for SCLC and the efficacy of perioperative chemotherapy have not yet been determined. Because early-stage SCLC is a rare presentation, accounting for 2.4% to 3.4% of resected lung cancer,[13] and a definite preoperative diagnosis of cell type as SCLC is rather difficult, studies prospectively comparing the significance of surgical resection in a large number of cases are unlikely to be conducted.

This study aimed to investigate recent surgical results for SCLC patients retrospectively, based on the large-volume Japanese nationwide registry database.

PATIENTS AND METHODS

Patients

In 2010, the Japanese Joint Committee of Lung Cancer Registry performed a nationwide retrospective registry study regarding the prognoses and clinicopathologic profiles of 11,663 patients who underwent resection for primary lung cancer in 2004. Of those patients, 243 with histologically confirmed SCLC (2.1%) were extracted from the database. The clinicopathologic factors and their relationship to postoperative survival were evaluated.

The following items were included for analysis: gender, age, smoking status, serum tumor markers (carcinoembryonic antigen and proGRP), clinical tumor, node, metastasis (TNM) stage (c-stage), pathological TNM stage (p-stage), surgical procedure, surgical curability (R0 and R1/R2), presence or absence of preoperative and postoperative chemotherapy, and survival time. The Union for International Cancer Control TNM staging, version 7,[14] was adopted in this study.

This study and the Japanese Joint Committee of Lung Cancer Registry registration study adhere to the Ethical Guidelines for Epidemiologic Research imposed by the Japanese Ministry of Health, Labor, and Welfare.[15]

Statistical Analysis

Survival time was defined as the time from the date of the surgery to the date of the last follow-up. Survival curves were estimated by the Kaplan-Meier method. Differences in survival were assessed by the log-rank test. A multivariate analysis by Cox's proportional hazards model was used to test the significance of prognostic factors. Statistical significance was considered to be established when the associated p value was less than 0.05.

A kappa value of conformity between c-stage and p-stage was also determined.[16] A kappa has a maximum of 1 (indicating perfect agreement) and a minimum −1 (indicating worse than chance agreement). A value of 0 indicates an agreement that is no better than chance, values above 0.4 are usually considered indicative of "moderate" agreement, and values higher than 0.6 are considered "good" agreement.

RESULTS

Patient Profiles

The clinicopathologic characteristics of the 243 patients with resected SCLC are summarized in Table 1. Of the 243 patients with resected SCLC, there were 213 (87.7%) men and 30 (12.3%) women. The mean age at the time of operation was 68.2±9.5 years. Preoperative serum proGRP levels were elevated in 58 patients (23.9%) and within normal limits in 185 (76.1%) patients. The major operative mode was lobectomy/bilobectomy (n = 174, 71.6%), followed by segmentectomy/wedge resection (n = 51, 21.0%). More than 60% of patients (n = 169, 68.6%) were diagnosed as c-stage IA or IB. As for the pathologic stage, 93 patients (38.3%) were recognized as p-stage IA, and 51 (21.0%) as p-stage IB. There were 45 (18.5%) patients in p-stage IIIA. Complete resections (R0) were achieved in 214 (88.1%) patients.

Postoperative Survival

The overall postoperative survival curve is shown in Figure 1. The 5-year survival rate of the 243 patients with SCLC was 52.6%. The postoperative survival curves according to c-stage and p-stage are shown in Figure 2. The 5-year survival rates by c-stage and p-stage were as follows: 64.3% in c-stage IA, 45.7% in c-stage IB, 50.5% in c-stage IIA, 33.3% in c-stage IIB, 30.5% in c-stage IIIA, 0% in c-stage IV, 72.3% in p-stage IA, 61.1% in p-stage IB, 44.8% in p-stage IIA, 40.3% in p-stage IIB, 23.4% in p-stage IIIA, and 0% in p-stage IV. The differences in survival were significant between c-stage IA and c-stage IB (p = 0.0423), c-stage IA and c-stage IIB (p = 0.0367), c-stage IA and IIIA (p = 0.0023), p-stage IA and p-stage IIA (p = 0.0074), p-stage IA and p-stage IIB (p = 0.0033), p-stage IA and p-stage IIIA (p = 0.0000), and p-stage IB and p-stage IIIA (p = 0.0006).

The relationship of each factor to survival, determined by univariate analysis, is shown in Table 1. Except for c-stage and p-stage, there was statistical significance in gender (women fared better than men did), serum ProGRP level (worse in elevated cases), and surgical curability (R0 patients fared better than R1/R2 patients did). In a Cox proportional hazards model to predict overall survival, the following factors persisted as significant prognostic factors: gender, age, c-stage, and surgical curability (Table 2).

Clinicopathological Results According to c-Stage

The relationship of p-stage, perioperative chemotherapy, and surgical curability to c-stage is shown in Table 3. In c-stage IA + IB, 39 of 168 cases (23.2%) were upstaged to p-stage, and eight of 30 cases (26.7%) in c-stage IIIA and two (66.7%) of three in c-stage IIIB were downstaged to p-stage I or II. A conformity of c-stage and p-stage was determined to be moderate, with a kappa value of 0.425.

As for surgical curability, in c-stage I (IA + IB), 158 cases (96.3%) underwent R0 resection and only six cases (3.7%) underwent R1/R2 resection. In c-stage II, 32 cases

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TABLE 1. Characteristics of Patients with Resected Small Cell Lung Cancer and Overall Survival

	N (%)	5-Year Survival (%)	Comparison	*p* Value
Gender				
Men	213 (87.7)	49.3		0.0190
Women	30 (12.3)	79.0		
Smoking				
Nonsmoker	22 (9.1)	41.6		
Ex-smoker	74 (30.5)	50.8	Nonsmoker vs. ex-smoker	0.5740
Smoker	124 (51.0)	56.3	Nonsmoker vs. smoker	0.2253
Unknown	23 (9.5)			
Operative mode				
Wedge resection	37 (15.2)	30.6	Wedge resection vs. lobectomy/bilobectomy	0.0019
Segmentectomy	14 (5.8)	63.6	Segmentectomy vs. lobectomy/bilobectomy	0.7848
Lobectomy/ bilobectomy	174 (71.6)	58.3		
Pneumonectomy	9 (3.7)	31.8	Pneumonectomy vs. lobectomy/bilobectomy	0.1600
Unknown	9 (3.7)			
c-stage				
IA	132 (54.3)	63.3		
IB	36 (14.3)	45.7	IB vs. IA	0.0423
IIA	25 (10.3)	50.5	IIA vs. IA	0.2531
IIB	10 (4.1)	33.3	IIB vs. IA	0.0367
IIIA	30 (12.3)	30.5	IIIA vs. IA	0.0023
IIIB	3 (1.2)	—	—	—
IV	7 (2.9)	0	IV vs. IA	0.0000
p-stage				
IA	93 (38.3)	72.3		
IB	51 (21.0)	61.1	IB vs. IA	0.1855
IIA	27 (11.1)	44.8	IIA vs. IA	0.0074
IIB	17 (7.0)	40.3	IIB vs. IA	0.0033
IIIA	45 (18.5)	23.4	IIIA vs. IA	0.0000
IIIB	1 (0.4)	—	—	—
IV	9 (3.7)	0	IV vs. IA	0.0000
Preoperative treatment				
Done	27 (11.1)			
None	215 (88.5)			
Unknown	1 (0.4)			
Adjuvant chemotherapy				
Done	158 (65.0)	52.0		0.5535
None	69 (28.4)	51.8		
Unknown	16 (6.6)			
Tumor marker				
CEA higher level	70 (28.8)	49.1		0.5631
CEA normal level	173 (71.2)	53.9		
ProGRP higher level	58 (23.9)	36.0		0.0482
ProGRP normal level	185 (76.1)	57.2		
Residual tumor				
R0	214 (88.1)	57.0		0.0000
R1/R2	23 (9.5)	10.2		
Unknown	6 (2.5)			

(94.1%) underwent R0 resection and three cases (8.8%) underwent R1/R2. In c-stageIIIA, R0 resections were done in 19 cases (65.5%). The 5 year survival rates of the patients who underwent R0 resection with c-stage IA, c-stage IB, and c-stage II (IIA+IIB) were 65.4%, 51.6%, and 44.4%, respectively.

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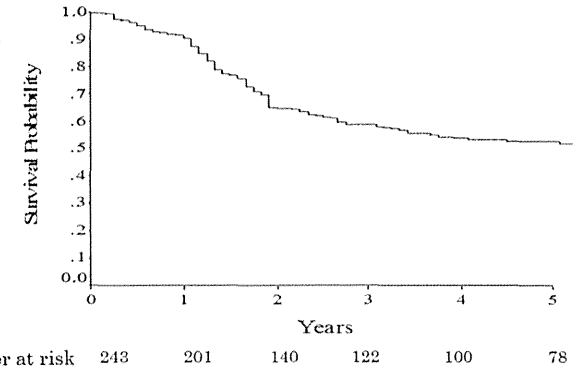

FIGURE 1. Overall survival curve. The 5-year survival rate of patients with small cell carcinoma was 52.6%.

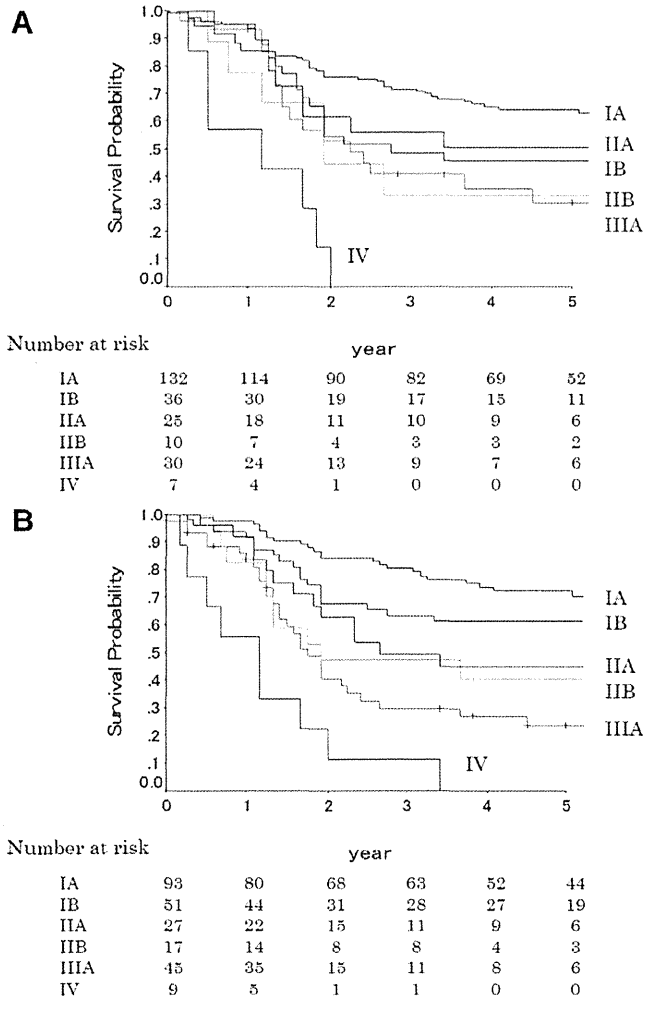

FIGURE 2. Overall survival curve based on clinical stage (Union for International Cancer Control-TNM Ver. 7). The 5-year survival rates by c-stage (*A*) and p-stage (*B*) were as follows: IA, 64.3% (n = 132) and 72.3% (n = 93); IB, 45.7% (n = 36) and 61.1% (n = 51); IIA, 50.5% (n = 25) and 44.8% (n = 27); IIB, 33.3% (n = 10) and 40.3% (n = 17); IIIA, 30.5% (n = 30) and 23.4% (n = 45); and IV, 0% (n = 7) and 0% (n = 9), respectively.

DISCUSSION

This study, which included 243 patients who underwent surgery in 2004, is the largest in number of patients with SCLC who underwent surgical intervention within just 1 year. It was expected that there would be low variations in preoperative staging evaluation, surgical technique, and postoperative care for each case. For such occasions, the results of this study were meaningful.

The current standard treatment for patients with SCLC is chemotherapy and radiotherapy, except for a portion of early-stage patients. The MRC study[3] in 1973 was a randomized trial, comparing surgery versus radiation alone. In that study, the median survival rate in the surgery group was 6.5 months, compared with 10 months in the radiation group (p = 0.04). After that article was published, the standard care was changed from surgical resection to radiotherapy. However, only 34 of the 71 patients (48%) who were enrolled in the surgery arm actually underwent surgical resection. Most of the patients in the MRC study had relatively advanced disease.

Recently, several authors have reported positive results for surgery in patients with early-stage SCLC.[17,18] Shah and colleagues[19] reported on surgical resection for SCLC patients without adjuvant chemotherapy in 1992. Of 28 patients who underwent surgical resection, 14 had stage I disease, five had stage II disease, and nine had stage III disease. The actual 5-year survival rate for patients in stage I was 57.1%, whereas no patients with stage II disease survived 5 years. In half of the patients in Shah's study, the tumor was in a central position. Lim and colleagues[11] reported excellent survival rates for patients in stages I to III who underwent lung resection with nodal dissection for SCLC. A total of 59 patients in their study underwent complete R0 resection for SCLC between 1980 and 2006, and the overall 5-year survival rate was 52%. That study supports the need to reevaluate surgery as the primary treatment and the use of clinical Tumor, Node, Metastasis criteria in the selection of patients with very limited SCLC for surgery. Weksler and colleagues[20] analyzed patients in the Surveillance Epidemiology and End Results database, making a retrospective analysis of a large national database. That study examined 3566 patients with stage I or II SCLC who underwent surgery from 1988 to 2007. Patients with stage II SCLC who had a lung resection had a median survival time of 25.0 months, compared with 14.0 months in patients with stage II SCLC who did not undergo lung resection ($p < 0.0001$). Weksler's study concluded that surgical resection as a component of treatment for stage I or stage II SCLC is associated with significantly improved survival and should be considered in the management of early-stage SCLC.

The overall 5-year survival rate of the patients in our study was 52.6%. Multivariate analysis found that good prognostic factors for survival were younger age, female gender, early-stage disease, and achieved curative resection. The same trends have been previously reported.[11,20] Even though c-stage was one of the most important prognostic factors, the survival rate of the selected patients with c-stage II was favorable results. In particular, patients who underwent complete resection had good survival rates, not only with c-stage I, but also with c-stage II, compared with previous reports. In c-stage

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I and II, 190 patients (95.5%) underwent R0 resection, and only nine underwent R1/R2 resection. Surgery was recommended for the c-stage I SCLC patients; however, based on these results, surgical resection might also be considered for patients with stage II SCLC.

On the other hand, in several patients in this study, c-stage did not correspond well with p-stage. Among the patients with stage I SCLC according to preoperative evaluations, 23.2% of the cases were upstaged to stage II or stage III postoperatively. A kappa value demonstrated moderate conformity between c-stage and p-stage in all cases. Vallieres and colleagues[10] reported the same trend when comparing clinical and pathological staging of SCLC, using the International Association for the Study of Lung Cancer database. The overall concordance between clinical and pathologic TNM staging was 58%. When grouping clinical stages I and II together, 19.7% were upstaged to stage pIIIA or above after resection according to the International Association for the Study of Lung Cancer database. Although there is no data on preoperative staging modality in the current study, intensive staging before considering surgical therapy is important, using such tools as positron emission tomography-computed tomography (PET-CT),[21] endobronchial ultrasound-guided transbronchial needle aspiration (EBUS-TBNA),[22] and surgical mediastinoscopy.[23]

Large cell neuroendocrine carcinoma (LCNEC) of the lung is defined as a high-grade neuroendocrine tumor no less than SCLC in the 1999 World Health Organization classification of lung tumors.[24] Takei et al.[25] reported that 44% (24 of 55) of operated patients who were originally diagnosed with SCLC (before 1999) were reclassified as LCNEC after the pathologic review. Studies on treatment of patients with SCLC naturally included many cases of LCNEC before LCNEC had been recognized. Thus, it is necessary to be aware when comparing studies performed before and after LCNEC was defined. The subjects of the current study are patients who were operated on in 2004, when LCNEC was well recognized.

In the present study, the survival benefit of postoperative adjuvant chemotherapy was not proved. It is assumed that because of biases in treatment acceptance, the patients' backgrounds were varied, although an analysis was conducted only in p-stage I patients.

Limitations of this analysis include that it is a retrospective study; there is no randomization for adjuvant treatment; there is a lack of preoperative histopathological diagnosis data; there is a lack of information regarding preoperative staging methods; and there is no information regarding the aim of the preoperative treatment and whether the induction treatment was followed by surgery or salvage surgery.

TABLE 2. Multivariate Analysis of Overall Survival for Resected Small Cell Lung Cancer; Cox Proportional Hazards Model

	Hazard Ratio	95% CI	*p* Value
Age, per year increase	1.038	1.015–1.062	0.001
Gender			
Men	1.00		
Women	0.356	0.142–0.893	0.028
c-stage			0.029
IA	1.00		
IB	1.421	0.811–2.493	0.220
IIA	1.298	0.618–2.727	0.491
IIB	2.389	0.986–5.788	0.054
IIIA	1.514	0.797–2.876	0.205
IIIB	3.739	0.863–16.204	0.078
IV	4.557	1.769–11.741	0.002
Tumor marker			
ProGRP normal level	1.00		
ProGRP higher level	1.232	0.774–1.961	0.378
Residual tumor			
R0	1.00		
R1/R2	2.288	1.208–4.332	0.011

CI, confidence interval

CONCLUSION

Surgical resection for selected patients with early-stage SCLC, especially stage I, had good survival outcomes. Based on this result, surgery might also be considered in c-stage II SCLC. Further, a clinical trial on the surgery for patients with c-stage II SCLC was recommended.

TABLE 3. Relationships Between c-Stage, p-Stage, Surgical Curability, and Perioperative Treatment

		p Stage							Surgical Curability[a]	
		IA	IB	IIA	IIB	IIIA	IIIB	IV	R0	R1/2
c-stage	IA	80	23	10	8	11	0	0	126	3
	IB	4	21	3	1	6	0	1	32	3
	IIA	5	2	11	2	4	1	0	21	3
	IIB	0	2	1	4	2	0	1	10	0
	IIIA	4	2	0	2	21	0	1	19	10
	IIIB	0	1	1	0	0	0	1	3	0
	IV	0	0	1	0	1	0	5	3	4

R0, no residual tumor; R1/R2, microscopic or macroscopic residual tumor.
[a]Six patients data of curability were missing.

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Large-Cell Neuroendocrine Carcinoma of the Lung
Surgical Management

Hiroyuki Sakurai, MD*, Hisao Asamura, MD

KEYWORDS

• Non–small-cell lung cancer • Large-cell neuroendocrine carcinoma • Prognosis

KEY POINTS

- Large-cell neuroendocrine carcinoma (LCNEC) of the lung is an uncommon aggressive neoplasm with a poor prognosis compared with non–small-cell lung carcinoma (NSCLC).
- Because of its rarity, the treatment recommendations for LCNEC are not based on clinical trials, but are extrapolated from the approach to patients with NSCLC and small-cell lung carcinoma and the established literature for LCNEC, which is primarily retrospective in nature.
- Further studies should clarify the histology-specific characteristic and optimal therapeutic approach to establish the entity of LCNEC.

INTRODUCTION

Large-cell neuroendocrine carcinoma (LCNEC) of the lung is a relatively uncommon and aggressive subset of non–small-cell carcinomas (NSCLC), within the spectrum of pulmonary neuroendocrine tumors, which include typical and atypical carcinoid, and small-cell lung cancer (SCLC).[1–3] LCNECs were first reported by Travis and colleagues[4] in 1991 as a separate category of pulmonary neuroendocrine tumors, distinct from typical and atypical carcinoids and SCLC. They described LCNECs as tumors composed of large cells characterized by a light microscopic neuroendocrine appearance with a low nuclear-to-cytoplasmic ratio, frequent nucleoli, a high mitotic rate (greater than 10 mitoses per 10 high-power fields), and abundant necrosis, in addition to neuroendocrine differentiation detected by electron microscopy or immunohistochemistry.[4] In the 1999 World Health Organization (WHO) classification,[5] these pathologic features were adopted as criteria for the LCNEC diagnosis.

LCNEC of the lung is considered to be very aggressive, and clinical outcome is poorer than expected for stage-matched NSCLC, similar to the dismal outcome of SCLC, with 5-year survival rates ranging between 15% and 60%.[3,6–8] Therefore, considerable debate has emerged about whether these tumors should be treated or considered together with SCLC. However, the reported prognoses are heterogeneous and the optimum treatment has not yet been identified. Here we review the pertinent literature on resected LCNEC of the lung, and examine its clinicopathological features and prognosis.

CLINICAL CHARACTERISTICS

Although not well defined, the incidence of LCNEC in primary lung cancers is likely very low. Since Jiang and colleagues[9] reported that 22 (2.8%) of 766 resected primary lung cancers were classified as LCNEC, several investigators reported similar rates. Based on the available literature, the incidence of LCNEC among resected lung cancers appears to be between 2.1% and 3.5%.[10–12]

Division of Thoracic Surgery, National Cancer Center Hospital, 5-1-1 Tsukiji, Chuo-ku, Tokyo 104-0045, Japan
* Corresponding author.
E-mail address: hsakurai@ncc.go.jp

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Men most commonly comprise 80% to 90% of patients with LCNEC[7,11,13]: Sarkaria and colleagues[14] reported 54% men in the Memorial LCNEC series. More than 85% of patients have a history of cigarette smoking[7,11,15]; therefore, smoking appears to be the primary cause in LCNEC development. The median age of patients ranged between 62 and 68 years.[7,12,14,16,17]

Regarding the tumor location, LCNECs mostly present as peripheral tumors,[3,18] as opposed to the central carcinoids and SCLC site, and therefore clinical symptoms are less commonly detected. Garcia-Yuste and colleagues[19] reported that two-thirds of LCNECs presented in the pulmonary parenchyma periphery. A computed tomography appearance generally shows a well-defined and lobulated nodule/mass that resembles that of other expansively growing tumors, such as peripheral SCLC, poorly differentiated adenocarcinomas, and squamous cell carcinomas (**Fig. 1**).[20–23] Regarding 2-[^{18}F]-fluoro-2-deoxy-D-glucose positron-emission tomography findings, Kaira and colleagues[24] reported that the standardized uptake value peek was significantly higher in LCNEC as well as SCLC, than in carcinoid, with a mean value of 13.7.[24]

Paraneoplastic and ectopic hormone production syndromes have been very infrequently observed and occasionally reported by Travis and colleagues,[25] Paci and colleagues,[18] Asamura and colleagues,[7] Takei and colleagues,[11] and Zacharias and colleagues.[26] Among serum tumor markers measured before surgery, the carcinoembryonic antigen and progastrin-releasing peptide, which is a good marker of high-grade neuroendocrine tumors such as SCLS, seem to be relatively elevated in patients with LCNEC (**Table 1**).[7,10,11]

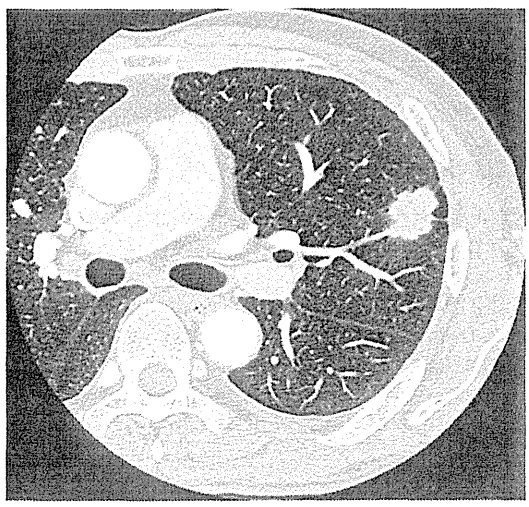

Fig. 1. Large-cell neuroendocrine carcinoma of the lung on high-resolution computed tomography. The tumor shows a well-defined lobulated nodule at the periphery in the left upper lobe of the lung.

Additionally, Sarkaria and colleagues[14] observed how LCNEC has a comparatively high incidence (21%) of prior nonlung cancers.

It is difficult to diagnose LCNECs from preoperative biopsies, although cytologic characteristics have been carefully studied.[27–29] LCNECs have been usually diagnosed in surgical specimens, postoperatively.

PATHOLOGY

Neuroendocrine tumors of the lung are a distinct subset of tumors that share definite morphologic, ultrastructural, immunohistochemical, and molecular characteristics.[30] Additionally, they encompass a spectrum of low-grade typical carcinoid, intermediate-grade atypical carcinoid, and high-grade LCNEC and SCLC.[25] Mitotic activity is the most important criterion to establish tumor type.

Immunohistochemical markers offer the most reliable means to detect neuroendocrine differentiation. Neuroendocrine tumors are identified by the presence of one or more of the following neuroendocrine markers: chromogranin A, synaptophysin, and neural cell adhesion molecule (NCAM).[5] Rossi and colleagues[31] first described the percentage of chromogranin A (65%), synaptophysin (53%), and NCAM (93%) in LCNEC. Evidence of neuroendocrine differentiation also can be ultrastructurally achieved through electron microscopy. Rusch and colleagues[32] identified the patterns of expression of several molecular markers in pulmonary neuroendocrine tumors. The investigators showed that Ki-67, p53, and Rb expression could be useful to distinguish LCNEC and SCLC from typical and atypical carcinoids.[32] Moreover, LCNEC and SCLC show a higher Ki-67 proliferation rate, abnormal p53, and the lack of Rb staining in comparison with typical and atypical carcinoids.[32]

Table 1
Percentage of preoperative elevation of serum tumor markers (CEA, NSE, and proGRP) for large cell neuroendocrine carcinoma

Author (Year)	CEA (%)	NSE (%)	proGRP (%)
Iyoda et al,[10] 2001	34.0	34.1	—
Takei et al,[11] 2002	49.0	19.0	11.0
Asamura et al,[7] 2006	48.5	12.4	25.8

Abbreviations: CEA, carcinoembryonic antigen; NSE, neuron-specific enolase; proGRP, progastrin-releasing peptide.

Data from Refs.[7,10,11]

Additional molecular analysis might elucidate a role of targeted therapies for LCNEC.[30]

Histologic LCNEC characteristics include large cell type (at least 3 times larger than SCLC), low nuclear-to-cytoplasmic ratio, high mitotic rate and necrosis, in addition to neuroendocrine morphology. The criteria for a correct LCNEC diagnosis, based on the recent WHO classification[5] are (1) neuroendocrine morphology (organoid nesting, palisading, rosettes, trabeculae), (2) high mitotic rate (11 or more per 2 mm^2 in 10 high-power fields), (3) necrosis, (4) cytologic features of a NSCLC, and (5) positive immunohistochemical staining for 1 or more neuroendocrine markers and/or neuroendocrine granules by electron microscopy. A preoperative diagnosis of LCNEC, based on small biopsies or cytology, is very difficult because of the problems identifying the neuroendocrine morphology and demonstrating neuroendocrine differentiation by immunohistochemistry in a small tissue sample.[8]

On the other hand, LCNEC is considered to be a variant of large-cell carcinoma according to the Third WHO classification.[5] Large-cell carcinomas (LCCs) are, in fact, divided into the following 4 types according to neuroendocrine features: (1) LCNEC, (2) large-cell carcinoma with neuroendocrine differentiation (LCCND), large-cell carcinoma with a neuroendocrine morphology (LCCNM), and classic large-cell carcinoma (CLCC).[5] The diagnostic criteria for these tumors are shown in Table 2. As reported by Iyoda and colleagues[10] on 199 cases of LCC, 50 (42%) were classified as LCNEC, 9 (7.6%) as LCCND, 13 (10.9%) as LCCNM, and 47 (39.5%) as CLCC. Additionally, the overall survival for patients with LCC with neuroendocrine features, such as LCNEC, LCCND, and LCCNM, was significantly shorter than that for patients with CLCC.[10] The clinical behavior of LCCND and LCCNM is likely to be similar to that of LCNEC.[28,33]

When LCNECs are accompanied by other histologies (squamous cell, spindle cell carcinoma) they are called combined LCNEC. Approximately 10% to 20% of surgically resected LCNECs are combined.[1,7,10] The commonest associated histologic subtype is adenocarcinoma.[10] If SCLC is coexistent with LCNEC, the tumor is diagnosed as a combined SCLC.[5]

SURVIVAL

The 5-year overall survival rate in patients with resected LCNEC has been reported to range between 13% and 57% for all stages,[34] and between 18% and 88% for patients with stage 1 (Table 3).[6,7,9–12,14,16,18,19,25,26,35–37] This difference is probably due to the small number of patients with LCNEC included in each report or an imprecise pathologic diagnosis of LCNEC. Asamura and colleagues[7] reported outcomes of surgically resected pulmonary neuroendocrine tumors including 141 LCNECs, the histologic diagnosis of which was reviewed by a pathology panel consisting of 6 expert pathologists. According to this report, the survival curves of patients with LCNEC and SCLC were superimposed and far worse than those of the patients with bronchial carcinoid, with 5-year survival rates of 40% and 36% for LCNEC and SCLC, compared with 96% and 78% for typical and atypical carcinoids. In many series, patients with resected LCNEC have worse survival compared with those with LCC or other NSCLCs,[12,38,39] even in stage 1.[12,37] LCNEC survival rates are almost similar to SCLC rates.[7,25,40–42] These 2 histologies share a similar clinicopathologic background, including smoking history and predominant male sex. Asamura and colleagues[7] found that an LCNEC histology was an independent predictor of a poor prognosis, as supported by other researchers.[12,17,37]

Combined LCNECs appear to behave as LCNEC rather than LCC.[8,14] Battafarano and

Table 2
Typing of large-cell carcinoma according to neuroendocrine features

Diagnosis	Neuroendocrine Morphology	Neuroendocrine Features on Immunohistochemistry or Electron Microscopy
LCNEC	Yes	Yes
LCCNM	Yes	No
LCCND	No	Yes
CLCC	No	No

Abbreviations: CLCC, classic large cell carcinoma; LCCND, large cell carcinoma with neuroendocrine differentiation; LCCNM, large cell carcinoma with neuroendocrine morphology; LCNEC, large cell neuroendocrine carcinoma.

Adapted from Lim E, Goldstraw P, Nicholson AG, et al. Proceedings of the IASLC international workshop on advances in pulmonary neuroendocrine tumors 2007. J Thorac Oncol 2008;3:1195; with permission.

Table 3
Postoperative 5-year survival rates for patients with large cell neuroendocrine carcinoma of the lung

Author (Year)	No. of Patients	5-Year Survival Rate (All) (%)	5-Year Survival Rate (Each Stage)
Dresler et al,[35] 1997	40	13.0	Stage 1 (n = 25), 18%
Travis et al,[25] 1998	37	27.0	—
Jiang et al,[9] 1998	17	44.8	—
Garcia-Yuste et al,[19] 2000	22	20.8	Stage 1, 33%
Iyoda et al,[10] 2001	50	35.3	—
Takei et al,[11] 2002	87	57.0	Stage 1 (n = 41)/2 (n = 13)/3 (n = 30), 67%/75%/45%
Skuladottir et al,[6] 2002	50	15.0	—
Zacharias et al,[26] 2003	21	47.0	Stage 1 (n = 9)/2–3 (n = 9), 88%/28%
Paci et al,[18] 2004	48	21.2	Stage 1 (n = 29)/2 (n = 11)/3 (n = 8), 27.0%/18.1%/0%
Doddoli et al,[36] 2004	20	36.0	Stage 1–2 (n = 8)/3–4 (n = 12), 54%/25%
Battafarano et al,[12] 2005	45	30.3	Stage 1 (n = 30), 33.3%
Iyoda et al,[37] 2006	11	—	Stage 1A (n = 11), 54.5%
Asamura et al,[7] 2006	141	40.3	Stage 1 (n = 63), 60%
Veronesi et al,[16] 2006	144	43.0	Stage 1 (n = 73)/2 (n = 29)/3 (n = 40), 52%/59%/20%
Sarkaria et al,[14] 2011	100	—	Stage 1A (n = 26)/1B (n = 18), 72%/26%

colleagues[12] showed that they behave poorly, with a 5-year overall survival rate of 30%. Therefore, the presence of neuroendocrine features in any portion of the tumor appears to be associated with poor prognosis. Additionally, Iyoda and colleagues[10] investigated the difference in the clinicobiological behavior of 4 LCC types (LCNEC, LCCND, LCCNM, and CLCC), based on their neuroendocrine features. The clinical behaviors of LCCNM and LCCND were similar to LCNEC, and these 3 types of LCC presented a worse outcome compared with CLCC.[10] Because LCC with any degree of neuroendocrine features shows a very aggressive biology, the pathology of LCC should be examined carefully for evidence of occult neuroendocrine features.

On a multivariate analysis, male gender, high age, advanced stage, and pneumonectomy have been reported to be negative prognostic factors.[1,14,16] According Faggiano and colleagues,[43] high mitotic count (>37 per 10 high-power fields) and less than 2 immunohistochemically positive neuroendocrine markers were independent negative pathologic variables. Nevertheless, Takei and colleagues[11] described that there was no correlation between the pattern of staining of neuroendocrine markers and survival.

Tumor recurrences usually develop early, even after a complete tumor resection. Iyoda and colleagues[17] reported that 64% of them occur within 1 year after surgery and 91% within 3 years; Takei and colleagues[11] observed 82% and 91% recurrences after 1 and 2 years, respectively. Most patients (56%–60%) present with distant metastases.[7,11,17]

TREATMENT

Treatment options for patients with LCNEC are based on the extrapolation from the approach to patients with NSCLC. Therefore, in stage 1 or 2 LCNEC, surgical resection is indicated whenever feasible. Because the prognosis is worse, adjuvant chemotherapy may improve survival, as suggested by several studies.[1,14,17] However, as a result of the small number of patients in each study and the relative infrequency of LCNEC, no standard adjuvant therapy regimen has been developed.[1,21] In a retrospective analysis of 83 patients, Rossi and colleagues[31] found significantly improved outcomes in those who received adjuvant SCLC-based therapy (cisplatin/etoposide) versus those who received platinum regimens in combination with other agents. In a small prospective, nonrandomized, single-arm trial, Iyoda and colleagues[44] reported 88.9% 5-year survival rate in patients receiving adjuvant cisplatin-etoposide versus 47.4% in those who did not. Disease-free survival and recurrence rates were also significantly better in patients who

received chemotherapy. This is the only prospective trial on adjuvant therapy, and the investigators concluded demonstrating the efficacy of adjuvant chemotherapy in such rare tumors.[44]

A recent meta-analysis showed that a cisplatin/irinotecan regimen may be an alternative to a cisplatin/etoposide regimen as first-line treatment in SCLC.[45,46] Thus, a prospective, randomized, multi-institutional phase III trial is currently being conducted by the Japan Clinical Oncology Group to compare cisplatin/etoposide to cisplatin/irinotecan in the setting of adjuvant chemotherapy for resected LCNEC. The role of neoadjuvant therapy has not yet been studied.[14]

Finally, there has also been a report on octreotide efficacy as adjuvant treatment in resected LCNEC.[47] Octreotide is a long-acting synthetic somatostatin analog that inhibits the secretion of a broad range of hormones, such as growth hormone, insulin, glucagon, and gastrin. Its antitumoral effect has been demonstrated in vitro[48] but the utility of octreotide in patients with LCNEC remains controversial.

SUMMARY

LCNEC of the lung is an uncommon aggressive neoplasm with a poor prognosis compared with NSCLC. Because of its rarity, the treatment recommendations are not based on clinical trials, but are extrapolated from the approach to patients with NSCLC and SCLC and the established literature for LCNEC, which is primarily retrospective in nature. Further studies should clarify the histology-specific characteristic and optimal therapeutic approach to establish the entity of LCNEC.

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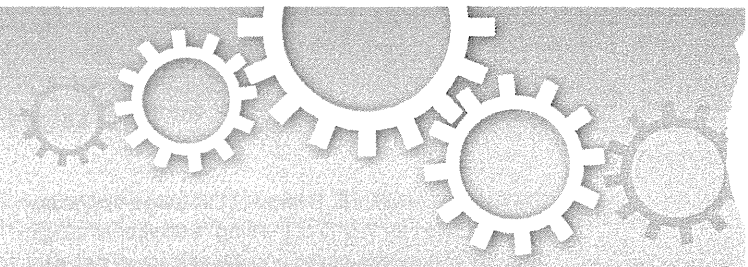

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NEK9-dependent proliferation of cancer cells lacking functional p53

Daisuke Kurioka[1,8], Fumitaka Takeshita[2], Koji Tsuta[5], Hiromi Sakamoto[3], Shun-ichi Watanabe[6], Kenji Matsumoto[7], Masatoshi Watanabe[8], Hitoshi Nakagama[4], Takahiro Ochiya[2], Jun Yokota[1,9], Takashi Kohno[1] & Naoto Tsuchiya[1]

[1]Division of Genome Biology, National Cancer Center Research Institute, [2]Division of Molecular and Cellular Medicine, National Cancer Center Research Institute, [3]Division of Genetics, National Cancer Center Research Institute, [4]Division of Cancer Development System, National Cancer Center Research Institute, [5]Division of Pathology and Clinical Laboratories, National Cancer Center Hospital, 5-1-1, Tsukiji, Chuo-ku Tokyo 104-0045, [6]Division of Thoracic Surgery, National Cancer Center Hospital, 5-1-1 Tsukiji, Chuo-ku Tokyo 104-0045, [7]Division of Allergy & Immunology, National Research Institute for Child Health and Development, 2-10-1 Okura Setagaya-ku Tokyo, 157-8535, [8]Laboratory for Medical Engineering, Division of Materials and Chemical Engineering, Graduate School of Engineering, Yokohama National University, 79-1 Tokiwadai, Hodogaya-ku Yokohama 240-8501, Japan, [9]Institute of Predictive and Personalized Medicine of Cancer (IMPPC), Barcelona, Spain.

Dysfunction of the p53 network is a major cause of cancer development, and selective elimination of p53-inactivated cancer cells therefore represents an ideal therapeutic strategy. In this study, we performed a microRNA target screen that identified NEK9 (NIMA-related kinase 9) as a crucial regulator of cell-cycle progression in p53-inactivated cancer cells. NEK9 depletion selectively inhibited proliferation in p53-deficient cancer cells both *in vitro* and *in vivo*. The resultant cell-cycle arrest occurred predominantly in G1 phase, and exhibited senescence-like features. Furthermore, NEK9 repression affected expression of a broad range of genes encoding cell-cycle regulators and factors involved in mRNA processing, suggesting a novel role for NEK9 in p53-deficient cells. Lung adenocarcinoma patients with positive staining for NEK9 and mutant p53 proteins exhibited significantly poorer prognoses, suggesting that expression of both proteins promotes tumor growth. Our findings demonstrate that a novel NEK9 network regulates the growth of cancer cells lacking functional p53.

The p53 tumor-suppressor pathway is the most important cellular network involved in preventing transformation of normal cells following exposure to various oncogenic insults[1]. In response to oncogenic stress, p53 activation leads to cell-cycle arrest, allowing for repair of damage and survival, or apoptosis, allowing for elimination of damaged cells, through stimulus-dependent transactivation of its target genes[2]. Cancer genome sequencing studies have shown that *TP53* is one of the genes most frequently mutated in human cancers[3,4]. Thus, dysfunction of the p53 signaling pathway(s) is a major cause of tumor onset and/or progression in almost all human cancers[5]. Furthermore, the molecular network(s) specifically activated in p53-deficient contexts may promote proliferation of cancer cells. Several lines of evidence strongly suggest that *TP53* mutations contribute to maintenance of the malignant gain-of-function phenotypes of cancer cells, including cell-cycle progression and activation of cell migration, as well as loss of wild-type tumor-suppressor functions[6]. In light of this novel concept, mutant p53 is an attractive target for therapeutics directed against a wide range of cancers.

MicroRNAs (miRNAs) are small non-coding RNAs that regulate gene expression at the post-transcriptional level[7]. Dysfunction of miRNAs is deeply involved in cancer development[8]. Many miRNAs are oncogenic and/or tumor-suppressive factors[9]; in particular, several miRNAs serve as intrinsic mediators that coordinate the p53 tumor-suppressor network in response to oncogenic stresses[10]. Each miRNA represses expression of a distinct set of target genes, determined in part by cellular characteristics influenced by p53 mutation status; consequently, these miRNAs induce different phenotypes in different cancer cells[11–14]. Thus, by exploiting their ability to affect the regulation of specific oncogenic or tumor-suppressive networks, individual miRNAs can be used as screening tools to identify therapeutic molecular targets in cancer cells.

Previously, we showed that *miR-22*, which acts on the p53 network, induces cell death in cancer cells with wild-type p53. On the other hand, it induces cell-cycle arrest in p53-mutant cancer cells that express CDK6, CDK3, SIRT1, and HDAC4, which are critical factors involved in cell-cycle progression and potential targets of *miR-22*[15]. This finding led us to hypothesize that *miR-22* has a unique set of target genes that determine the fate of cancer