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