Genomic Profiling and Clinicopathological Characteristics of Neuroendocrine Tumors of the Lung in East Asian Patients

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Abstract. *Background/Aim:* In recent years, the genomic landscape of neuroendocrine tumors (NETs) of the lung has been investigated. However, more data are necessary to elucidate the heterogeneous nature of NETs, especially in East Asian patients. *Patients and Methods:* A total of 64 patients who underwent surgical resection for lung NETs [26 typical or atypical carcinoid tumors, 21 large-cell neuroendocrine carcinomas (LCNECs), and 19 small-cell lung carcinomas (SCLCs)] were enrolled, and samples from 46 patients were subjected to targeted next-generation sequencing. *Results:* Co-mutations of tumor protein p53 (TP53) and RB transcriptional corepressor 1 (RB1) were detected in 15%, 42%, and 93% of carcinoid tumors, LCNECs, and SCLCs, respectively. Oncogenic or targetable genetic alterations identified in this study included mutations of KRAS proto-oncogene (KRAS), phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha (PIK3CA), ALK receptor tyrosine kinase (ALK), mitogen-activated protein kinase kinase 1 (MAP2K1), and isocitrate dehydrogenase 1 (IDH1), as well as amplifications of erb-b2 receptor tyrosine kinase 2 (ERBB2), fibroblast growth factor receptor 1 (FGFR1), CD274 molecule (CD274), and MYCN proto-oncogene (MYCN). These alterations were more frequently found in high-grade NETs than in carcinoid tumors (33.3% vs. 7.7%). Programmed cell death-ligand 1 expression was strongly associated with the LCNEC subtype among NETs ($p=0.002$). *Conclusion:* The mutational status of TP53 and RB1 was significantly associated with NET subtypes in East Asian patients. Targeted therapy or

immunotherapy may serve as a treatment option in a subset of patients with high-grade NETs.

Neuroendocrine tumors (NETs) of the lung are currently categorized based on their histological features. According to the 2015 World Health Organization (WHO) Classification of Lung Tumors and the International Agency for Research on Cancer (IARC) and WHO expert consensus proposal for neuroendocrine neoplasms (1, 2), lung NETs can be categorized into low-grade [typical carcinoid tumors (TCs)], intermediate-grade [atypical carcinoid tumors (ACs)], and high-grade [large-cell neuroendocrine carcinomas (LCNECs) and small-cell lung carcinomas (SCLCs)] tumors. They are characterized by histological features of neuroendocrine morphology (organoid nesting, palisading, or rosette-like structures) and express immunohistochemical neuroendocrine markers. TCs usually present with a centrally located mass involving the bronchi. Microscopically, they comprise monotonous cells with a granular cytoplasm that forms characteristic nest, ribbon, or fustoon patterns. The morphology of ACs is similar to that of TCs; however, they also demonstrate increased mitotic activity (2-10 mitoses per 2 mm²) with/without the presence of necrosis. Of the high-grade NETs, LCNEC is a type of non-small-cell lung carcinoma (NSCLC) that displays abundant cytoplasm and prominent nucleoli with neuroendocrine differentiation. SCLC consists of small cells with scant cytoplasm, a fine granular chromatin pattern, and inconspicuous nucleoli. High-grade NETs typically show high mitotic activity (>10 mitoses per 2 mm²) and an increased Ki-67 cell proliferation labeling index (>40%) (3).

In recent years, comprehensive genomic profiling of lung NETs has been reported (4-10). In addition to morphological classification, recent studies have suggested that NETs consist of heterogeneous tumors despite sharing a similar morphology (4, 5, 7, 8, 11-16). They can be further subdivided into molecular subtypes based on genomic characteristics (4, 5, 7, 8, 11, 15, 16).

However, most of the existing research on lung NETs has focused on Western populations. Multiple studies have demonstrated that ethnicity plays a role in the prevalence of important genetic markers in NSCLC (17). Therefore, it is

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worthwhile investigating the genomic profiling of lung NETs in Asian populations. In addition to the genetic analyses of lung NETs, recent studies have also suggested that high-grade neuroendocrine carcinomas may be associated with a distinct tumor immune microenvironment compared to carcinoid tumors (18-22). In this study, we investigated the genetic profiling, clinicopathologic features, and programmed cell death-ligand 1 (PD-L1) status of lung NETs in East Asian patients.

Patients and Methods

Patients. Patients who underwent surgical resection for NETs of the lung (including TCs, ACs, LCNECs, and SCLCs) between 2005 and 2012 were enrolled in this study. All cases were chemo-naïve. Detailed clinical data, including age, sex, smoking history, stage, and survival, were retrieved from medical records. Tumor stage was determined according to the eighth edition of the American Joint Committee on Cancer (AJCC) staging manual (23). The Institutional Review Board approved this study (No. 4-2017-0206). Written informed consent from the patients was waived due to the retrospective nature of the study.

Pathological evaluation. Two pathologists (MK and HSS) who specialize in lung pathology reviewed all of the available hematoxylin and eosin-stained or immunohistochemically stained slides. All diagnoses were made according to the 2015 WHO classification of lung tumors (1). Discrepancies in classification were resolved by consensus discussion. Pathological findings, including mitoses, necrosis, and invasion to lymphovascular spaces or pleura, were recorded.

Immunohistochemistry (IHC) and interpretation. A representative tumor section was stained with antibodies against cytokeratin AE1/AE3 (mouse monoclonal, clone AE1/AE3, 1:600; Agilent, Santa Clara, CA, USA), synaptophysin (mouse monoclonal, clone DAK-SYNAP, 1:900; Agilent), chromogranin A (mouse monoclonal, clone DAK-A3, 1:500; Agilent), CD56 (mouse monoclonal, clone CD564, 1:100; Novocastra, Newcastle, UK), human epidermal growth factor receptor 2 (PATHWAY anti-HER-2/neu (4B5) Rabbit Monoclonal Primary Antibody; Ventana Medical Systems, Tucson, AZ, USA), and Ki-67 (mouse monoclonal, clone MB-1, 1:150; Agilent) using the Ventana BenchMark XT Automated Staining System (Ventana Medical Systems). Positive and negative controls were developed for the assessment of staining quality. For PD-L1 22C3 IHC, the sections were stained using an Autostainer Link 48 platform (Agilent) following the manufacturer's instructions. The tumor proportion score (TPS) was determined as the percentage of tumor cells showing membranous positivity to PD-L1 antibody regardless of the staining intensity. A TPS $\geq 1\%$ was defined as PD-L1 positivity.

Targeted DNA and RNA next-generation sequencing (NGS). Targeted DNA and RNA sequencing were performed using a customized cancer panel (NGeneBio, Seoul, Republic of Korea). The customized cancer panel was designed to detect 46 cancer-related genes, including 46 genes with potential single nucleotide variants (SNVs) and insertions and deletions (indels), 20 genes with potential amplification, and 17 genes with potential fusion variants (Table I). Briefly, representative sections containing at least 30% tumor volume were selected. Formalin-fixed, paraffin-embedded (FFPE) tissue-derived DNA and RNA were extracted using QIAGEN AllPrep DNA/RNA FFPE Kit

Table I. Genes targeted in the next-generation sequencing panel.

Gene	Encoded protein	SNVs & indel (DNA)	Amplification (DNA)	Fusion (RNA)
AKT1	AKT1	x	x	x
ALK	ALK	x	x	
ARAF	ARAF	x		
BRAF	BRAF	x	x	x
BRCA1	BRCA1	x		
BRCA2	BRCA2	x		
CD274	PD-L1	x	x	
CRKL	CRKL	x	x	
DDR2	DDR2	x		
EGFR	EGFR	x	x	x
ERBB2	HER2	x	x	x
ERBB4	ERBB4	x		x
EZH2	EZH2	x		
FGFR1	FGFR1	x	x	x
FGFR2	FGFR2	x	x	x
FGFR3	FGFR3	x	x	x
IDH1	IDH1	x		
IDH2	IDH2	x		
JAK1	JAK1	x		
JAK2	JAK2	x		
JAK3	JAK3	x		
KIT	KIT	x	x	
KRAS	KRAS	x	x	
MAP2K1	MAP2K1	x		
MET	HGFR	x	x	x
MTOR	mTOR	x		
MYC	MYC	x	x	x
MYCN	N-myc	x	x	
NRAS	NRAS	x		
NRG1	NRG1	x		x
NTRK1	TrkA	x		x
NTRK2	TrkB	x		x
NTRK3	TrkC	x		x
PDGFRA	PDGFRA	x	x	x
PIK3CA	PIK3CA	x	x	
PTEN	PTEN	x		
RB1	Rb	x		
RET	RET	x	x	x
RICTOR	RICTOR	x	x	
RIT1	RIT1	x		
ROS1	ROS1	x	x	x
SMARCA4	BRG1	x		
STK11	STK11	x		
TP53	p53	x		
TSC1	TSC1	x		
TSC2	TSC2	x		

SNV: Single nucleotide variant; indel: insertion and deletion.

(Qiagen, Hilden, Germany). After hybridization capture-based target enrichment using 100 ng of DNA and 500 ng of RNA, paired-end sequencing (2×150 bp) was performed using a MiSeq sequencer (Illumina, San Diego, CA, USA) according to the manufacturer's instructions. SNVs, indels, and copy number variations were analyzed using the Burrows-Wheeler Aligner and Genome Analysis Tool Kit. Variants with a total depth of at least 100× and a variant allele

Figure 1. Representative photos of lung neuroendocrine tumors according to subtype. A: typical carcinoid tumor, B: atypical carcinoid tumor, C: large-cell neuroendocrine carcinoma, and D: small-cell lung carcinoma (original magnifications: A: $\times 100$; B-D: $\times 200$).

frequency of at least 3% were included for analysis. Fusion variants were determined using STAR-Fusion and FusionCatcher. Variant interpretation was based on recommendations from the Association for Molecular Pathology, the American Society of Clinical Oncology, and the College of American Pathologists (24).

Statistical analysis. Chi-square tests were used to demonstrate the relationships between clinicopathological and categorical parameters. Recurrence-free (RFS) and overall (OS) survival were investigated using the Kaplan-Meier method. To determine statistical differences between survival times, the log-rank test was performed. A p -value of less than 0.05 was considered as statistically significant. All statistical calculations were conducted using IBM SPSS Statistics 23 software (IBM, Armonk, NY, USA).

Results

Clinicopathologic characteristics of NETs. A total of 64 patients who underwent complete surgical resection and were diagnosed with primary lung NETs were enrolled in this study.

Fifteen cases (23.4%) were classified as TCs, nine (14.0%), as ACs, 21 (32.9%), as LCNECs, and 19 (29.7%), as SCLCs (Figure 1). Clinicopathological characteristics according to NET subtype are summarized in Table II. Of the 64 patients, 45 patients (70.3%) were male, and 19 (29.7%) were female. The mean age was 61.2 years, and the age ranged from 24 to 78 years. There were significant differences in age ($p < 0.001$), gender ($p = 0.001$), smoking history ($p < 0.001$), tumor size ($p = 0.031$), pleural invasion ($p = 0.011$), lymphovascular invasion ($p = 0.001$), lymph node metastasis ($p = 0.003$), and pathologic stages ($p = 0.011$) among the subtypes. In terms of survival, the median period of follow-up was 38 months at the time of analysis. Kaplan-Meier analyses for overall OS and RFS demonstrated significant differences according to NET subtype ($p = 0.005$ and $p = 0.004$, respectively; Figure 2).

Overview of the genomic profiling of NETs. We then investigated the genomic profiling of a total of 46 NETs

Table II. Patient characteristics according to the lung neuroendocrine tumor subtype.

Variable	All (n=64)	TC (n=15)	AC (n=9)	LCNEC (n=21)	SCLC (n=19)	p-Value
Age (mean)	61.2±11.2	55.1±14.6	51.9±10.9	65.4±6.5	65.8±7.5	<0.001
Gender						0.001
Male	45 (70.3%)	7 (46.7%)	3 (33.3%)	18 (85.7%)	17 (89.5%)	
Female	19 (29.7%)	8 (53.3%)	6 (66.7%)	3 (14.3%)	2 (10.5%)	
Smoking						<0.001
Ever	45 (70.3%)	7 (46.7%)	2 (22.2%)	19 (90.5%)	17 (89.5%)	
Never	19 (29.7%)	8 (53.3%)	7 (77.8%)	2 (9.5%)	2 (10.5%)	
Tumor size	2.7±1.5	1.7±0.5	2.6±1.7	3.4±1.7	2.7±1.6	0.031
PL						0.011
Present	12 (18.7%)	0 (0.0%)	0 (0.0%)	8 (38.1%)	4 (21.1%)	
Absent	52 (81.3%)	15 (100.0%)	9 (100.0%)	13 (61.9%)	15 (78.9%)	
LVI						0.001
Present	23 (35.9%)	1 (6.7%)	0 (0.0%)	10 (47.6%)	12 (63.2%)	
Absent	41 (64.1%)	14 (93.3%)	9 (100.0%)	11 (52.4%)	7 (36.8%)	
LN metastasis						0.003
Present	26 (40.6%)	4 (26.7%)	0 (0.0%)	9 (42.9%)	13 (68.4%)	
Absent	38 (59.4%)	11 (73.3%)	9 (100.0%)	12 (57.1%)	6 (31.6%)	
TNM stage						0.011
Stage I	31 (48.4%)	11 (73.3%)	8 (88.9%)	7 (33.3%)	5 (26.3%)	
Stage II	17 (26.6%)	1 (6.7%)	1 (11.1%)	7 (33.3%)	8 (42.1%)	
Stage III	16 (25.0%)	3 (20.0%)	0 (0.0%)	7 (33.3%)	6 (31.6%)	

AC: Atypical carcinoid tumor; LCNEC: large-cell neuroendocrine carcinoma; LN: lymph node; LVI: lymphovascular invasion; PL: pleural invasion; SCLC: small-cell lung carcinoma; TC: typical carcinoid tumor.

(seven TCs, six ACs, 19 LCNECs, and 14 SCLCs) using targeted NGS. Cases were selected on the basis of archival FFPE tissue availability for NGS analysis. There were no differences in clinicopathological parameters between the whole cohort (n=64) and the subset analyzed by NGS (n=46). The mean depth was 390[×]. Genetic alterations according to NET subtype are summarized in Figure 3 and Table III. A total of 77 nonsynonymous mutations (four in TCs, four in ACs, 34 in LCNECs, and 35 in SCLCs) and five copy number gains (four in LCNECs and one in SCLCs) were identified. RNA fusions were not detected. Co-mutations of tumor protein p53 (*TP53*) and RB transcriptional corepressor 1 (*RBI*) were detected in 15.4% (2/13), 42.1% (8/19), and 92.9% (13/14) of carcinoid tumors, LCNECs, and SCLCs, respectively. Oncogenic or targetable genetic alterations identified in this study included mutations of KRAS proto-oncogene (*KRAS*), phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha (*PIK3CA*), ALK receptor tyrosine kinase (*ALK*), mitogen-activated protein kinase kinase 1 (*MAP2K1*), and isocitrate dehydrogenase 1 (*IDH1*), as well as amplifications of erb-b2 receptor tyrosine kinase 2 (*ERBB2*), fibroblast growth factor receptor 1 (*FGFR1*), CD274 molecule (*CD274*), and MYCN proto-oncogene (*MYCN*). These alterations were more frequently found in high-grade NETs than in carcinoid tumors (33.3% vs. 7.7%).

Genetic alterations in TCs and ACs. In TCs, *TP53* (2/7, 28.6%) and *RBI* (2/7, 28.6%) were rarely identified. *TP53* and *RBI* co-mutation was detected in only one case, and pathological review confirmed the TC diagnosis. The Ki-67 labeling index for this case was less than 2%. Interestingly, this case showed lymph node metastasis at the time of diagnosis. However, there was no recurrence over an 8-year follow-up period after surgical resection.

In ACs, mutations of *TP53*, *RBI*, and phosphatase and tensin homolog (*PTEN*) were simultaneously found in one case. During follow-up, the patient with this tumor died due to another cancer (advanced renal cell carcinoma). Among the targetable oncogenes, a *PIK3CA* (p.H1047L) mutation was observed in one case (1/6, 16.7%).

Genetic alterations in LCNECs. *TP53* (16/19, 84.2%), *RBI* (9/19, 42.3%), *PTEN* (3/19, 15.8%), and SWI/SNF related, matrix associated, actin dependent regulator of chromatin, subfamily a, member 4 (*SMARCA4*) (2/19, 10.5%) were identified in LCNECs. All mutations in the *TP53* gene affected a critical functional DNA-binding domain. The majority of *RBI* mutations (88.9%) were nonsense or frameshift variants. No serine/threonine kinase 11 (*STK11*) mutations were identified. Amplifications of *ERBB2*, *MYCN*, *FGFR1*, and *CD274* were each detected in one case (1/19, 5.3%). Expression and gene amplification of *ERBB2* were

Figure 2. Survival curves according to the lung neuroendocrine tumor subtype. A: overall survival ($p=0.005$), B: recurrence-free survival ($p=0.004$).

Figure 3. OncoPrint heatmap for genetic alterations in lung neuroendocrine tumors according to subtype. Please refer to Table I for gene designations. AC: Atypical carcinoid tumor; LCNEC: large-cell neuroendocrine carcinoma; SCLC: small-cell lung carcinoma; TC: typical carcinoid tumor.

confirmed by IHC and silver *in situ* hybridization, respectively (Figure 4A and B). We then categorized LCNECs into ‘NSCLC-like’ or ‘SCLC-like’ subtypes according to the *TP53* and *RB1* co-mutation status, which is

a defining event in SCLC. Notably, all the oncogenic alterations, including mutations of *KRAS* (p.G12V) and *IDH1* (p.R132C) and amplifications of *ERBB2*, *MYCN*, *FGFR1*, and *CD274*, were found in the ‘NSCLC-like’

Table III. Incidence of frequent (more than 1) genetic alterations in lung neuroendocrine tumors and their distributions according to the tumor subtype.

Gene	All (n=46)	TC (n=7)	AC (n=6)	LCNEC (n=19)	SCLC (n=14)	p-Value
TP53						<0.001
Mutation	32 (69.6%)	2 (28.6%)	1 (16.7%)	16 (84.2%)	13 (92.9%)	
Wild-type	14 (30.4%)	5 (71.4%)	5 (83.3%)	3 (15.8%)	1 (7.1%)	
RB1						0.003
Mutation	25 (54.3%)	2 (28.6%)	1 (16.7%)	9 (47.4%)	13 (92.9%)	
Wild-type	21 (45.7%)	5 (71.4%)	5 (83.3%)	10 (52.6%)	1 (7.1%)	
TP53+RB1						0.001
Co-mutation	23 (50.0%)	1 (14.3%)	1 (16.7%)	8 (42.1%)	13 (92.9%)	
Wild-type	23 (50.0%)	6 (85.7%)	5 (83.3%)	11 (57.9%)	1 (7.1%)	
PTEN						0.238
Mutation	9 (19.6%)	0 (0.0%)	1 (16.7%)	3 (15.8%)	5 (35.7%)	
Wild-type	37 (80.4%)	7 (100.0%)	5 (83.3%)	16 (84.2%)	9 (64.3%)	
SMARCA4						0.396
Mutation	2 (4.3%)	0 (0.0%)	0 (0.0%)	2 (10.5%)	0 (0.0%)	
Wild-type	44 (95.7%)	7 (100.0%)	6 (100.0%)	17 (89.5%)	14 (100.0%)	
IDH1						0.827
Mutation	2 (4.3%)	0 (0.0%)	0 (0.0%)	1 (5.3%)	1 (7.1%)	
Wild-type	44 (95.7%)	7 (100.0%)	6 (100.0%)	18 (94.7%)	13 (92.9%)	
PIK3CA						0.189
Mutation	3 (6.5%)	0 (0.0%)	1 (16.7%)	0 (0.0%)	2 (14.3%)	
Wild-type	44 (93.5%)	7 (100.0%)	5 (83.3%)	19 (100.0%)	12 (85.7%)	
MYCN						0.827
Amplification	2 (4.3%)	0 (0.0%)	0 (0.0%)	1 (5.3%)	1 (7.1%)	
No amplification	44 (95.7%)	7 (100.0%)	6 (100.0%)	18 (94.7%)	13 (92.9%)	

AC: Atypical carcinoid tumor; LCNEC: large-cell neuroendocrine carcinoma; SCLC: small-cell lung carcinoma; TC: typical carcinoid tumor. Please refer to Table I for gene designations.

subgroup, except for one case of ‘SCLC-like’ LCNEC with a *MAP2K1* (p.K57N) mutation. However, there were no statistically significant differences in clinicopathological parameters between the ‘NSCLC-like’ and ‘SCLC-like’ subtypes. In addition, OS did not demonstrate a statistically significant difference (Figure 5).

Genetic alterations in SCLCs. Mutations of *TP53* (13/14, 92.8%), *RB1* (13/14, 92.8%), and *PTEN* (5/14, 35.7%) were frequently identified in SCLCs. *MYCN* amplification was observed in one case (1/14, 7.1%). Co-mutation of *TP53* and *RB1* was found in all cases except one (13/14, 92.8%). A pathological review of the case harboring neither *TP53* nor *RB1* mutations confirmed the diagnosis of SCLC. The Ki-67 labeling index for this case was approximately 80%. Among oncogenic alterations, two *PIK3CA* mutations (p.E545K and p.G1049R), one *ALK* mutation (p.R1275Q), and one *IDH1* mutation (p.R132H) were identified.

Association of PD-L1 status with NETs. PD-L1 22C3 IHC was performed on samples from 64 NET cases (Table IV). PD-L1 positivity was more frequently observed in LCNECs than in the other NET subtypes ($p=0.002$). A case of LCNEC with *CD274* amplification showed TPS of 100% in PD-L1

IHC (Figure 4C). Upon further categorization of LCNECs into ‘NSCLC-like’ and ‘SCLC-like’ subtypes, there was no statistically significant difference in PD-L1 expression but ‘NSCLC-like’ LCNECs tended to display higher expression levels of PD-L1.

Discussion

In this study, we demonstrated that the mutational status of *TP53* and *RB1* is important for NET categorization in East Asian patients. There may also be opportunities for targeted therapy or immunotherapy in patients with NETs despite inter-tumor heterogeneity.

In this study, the frequency of *TP53* or *RB1* mutation was low in TCs and ACs, compared to that in high-grade NETs. One TC case harbored the co-mutation of *TP53* and *RB1* and showed lymph node metastasis at the time of diagnosis. However, there was no recurrence after complete surgical resection. More clinical case studies are needed to investigate the clinical significance of morphologically carcinoid tumors with co-mutations of *TP53* and *RB1*. The frequency of targetable genetic alterations was also very low in TCs and ACs. Previous studies observed frequent mutations in chromatin-remodeling genes and the menin 1

Figure 4. A case of large-cell neuroendocrine carcinoma (LCNEC) harboring amplifications of *erb-b2* receptor tyrosine kinase 2 (*ERBB2* or *HER2*) (A and B) and *CD274* (C). A: *HER2* immunohistochemical staining demonstrates diffuse membranous positivity. B: *ERBB2* silver in-situ hybridization illustrates the amplification of the *ERBB2* gene. C: A case of LCNEC with a *CD274* amplification with a 100% tumor proportion score in programmed cell death-ligand 1 (*PD-L1*) immunohistochemical staining.

(*MEN1*) gene (6, 10). The NGS gene panel used in this study was mainly designed for targetable genetic alterations in lung cancer; hence, we were unable to examine the frequency of mutations in the *MEN1* gene or chromatin-remodeling genes.

In this study, we also demonstrated that the co-mutational status of *TP53* and *RB1* can divide LCNECs into two subgroups. There was a clear distinction in terms of genetic alterations between ‘NSCLC-like’ and ‘SCLC-like’ LCNECs, which was consistent with previous studies (5, 8). Rekhtman *et al.* characterized ‘SCLC-like’ LCNECs as having a *TP53* and *RB1* co-mutation and ‘NSCLC-like’ LCNECs as lacking a *TP53* and *RB1* co-mutation, but retaining the presence of an NSCLC mutation, such as *STK11*, *KRAS*, and kelch like ECH associated protein 1 (*KEAP1*) (8). In our study, a *KRAS* mutation was identified in only one case of ‘NSCLC-like’ LCNEC, and no *STK* mutations were detected. This is likely

due to ethnicity-related variations in frequently altered genes. With regard to NSCLCs, previous studies have consistently reported lower rates of *KRAS* and *STK11* mutations in East Asian populations than in Western populations (17, 25, 26). This trend was also reported in a Japanese cohort of LCNECs (7). In LCNEC cases among Western populations, the frequency of *KRAS* mutations was 22% but in a Japanese cohort it was only 6% (7, 8).

For SCLCs, all cases except one harbored a *TP53* and *RB1* co-mutation, which is a key event in SCLC tumorigenesis. In addition, oncogenic mutations in *PIK3CA*, *IDH1*, and *ALK* were observed. Recent studies have indicated that SCLCs may consist of a heterogeneous subset, contradicting the traditional concept that SCLCs share a homogenous nature (11, 27, 28). SCLCs can be categorized depending on the expression of achaete-scute family bHLH

Table IV. Programmed cell death-ligand 1 (PD-L1) expression according to the lung neuroendocrine tumor subtype.

	All (n=64)	Carcinoid tumor (n=24)	LCNEC (n=21)	SCLC (n=19)	p-Value
PD-L1 TPS	8.7±22.9%	4.3±15.3%	20.6±34.1%	1.2±3.5%	0.037
PD-L1					0.001
Positive*	21 (32.8%)	3 (12.5%)	13 (61.9%)	5 (26.3%)	
Negative	43 (67.2%)	21 (87.5%)	8 (38.1%)	14 (73.7%)	

LCNEC: Large-cell neuroendocrine carcinoma; SCLC: small-cell lung carcinoma; TPS: tumor proportion score. *PD-L1 positivity was defined as TPS $\geq 1\%$.

transcription factor 1 (*ASCL1*), neuronal differentiation 1 (*NeuroD1*), Yes1 associated transcriptional regulator (*YAPI*), or POU class 2 homeobox 3 (*POU2F3*) (27, 28). Because we performed targeted NGS only, we were unable to correlate the genomic data with expression changes in *ASCL1*, *NeuroD1*, *YAPI*, or *POU2F3*. Further studies are necessary to investigate the clinical significance of the molecular subtypes of SCLC based on the transcriptional expression profiles. In this study, *PTEN* mutations were detected in five out of 14 SCLCs (36%). *PTEN* was suggested to be a potent suppressor of SCLC (29). The PIK3CA/*PTEN* pathway was altered in 43% of SCLCs in our cohort. This finding may implicate the PIK3 pathway inhibitors as a targeted therapy for a subset of patients with SCLC.

In terms of therapeutic potential, the detection of targetable mutations in high-grade NETs supports the necessity of NGS testing to identify the drug target, despite the relatively low frequency of these mutations. Since current treatment of unresectable NETs is largely based on platinum-based chemotherapy, detection of these genetic alterations might benefit patients by providing a novel therapeutic option (30, 31).

Cancer immunotherapy using immune checkpoint inhibitors (ICIs) has become a standard of care in the treatment of patients with lung cancer. IHC for PD-L1 is used as a predictive marker for response to ICIs (32). In this study, PD-L1 positivity was significantly related to high-grade NETs, especially LCNECs. This finding is consistent with previous studies (18-20). Kim *et al.* reported that the PD-1/PD-L1 pathway was activated in high-grade neuroendocrine carcinoma and may be related to higher mutational burdens (18). George *et al.* found that the expression of the components of immune-related pathways was increased in a subset of LCNECs, based on transcriptomic analysis (5). Given these findings, including those of our study, immunotherapy may serve as a treatment option for high-grade NETs, as previously described by the clinical response to ICIs (33, 34).

This study has several limitations. Since we applied an NGS panel that was mainly included well-known oncogene or tumor-suppressor genes, more recently described important genes in lung NETs were not included, such as

Figure 5. Overall survival according to the large-cell neuroendocrine carcinoma molecular subtype ($p=0.703$).

members of the *NOTCH* family, *KEAP1*, or *MEN1*. Limited sample numbers may also have affected the frequency of well-known driver mutations in lung NETs. Thus, subsequent large-scale studies are needed to confirm the genetic and clinicopathologic findings addressed in previous researches and the current work through the application of more detailed genomic analyses.

In conclusion, the mutational status of *TP53* and *RBI* was associated with NET subtypes, implying associations with tumorigenesis, biological behavior, and targetable genetic alterations. Although the frequency of such targetable alterations is found to be low, an NGS panel test is recommended because they were detected in high-grade NETs. In addition to these targetable mutations, our study also suggests that immunotherapy represents a viable treatment option for a subset of high-grade NETs.

Conflicts of Interest

The Authors declare no conflicts of interest.

Authors' Contributions

HSS and MK designed the study and wrote the initial draft of the article. All Authors contributed to data collection and interpretation, and critically reviewed the article. All Authors read and approved the final manuscript.

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