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Comprehensive Characterization of the Genomic Landscape in Chinese Pulmonary Neuroendocrine Tumors Reveals Prognostic and Therapeutic Markers (CSWOG-1901)

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Abstract

Background: Pulmonary neuroendocrine tumors (pNETs) include typical carcinoid (TC), atypical carcinoid (AC), large cell neuroendocrine carcinoma (LCNEC), and small cell lung carcinoma (SCLC). The optimal treatment strategy for each subtype remains elusive, partly due to the lack of comprehensive understanding of their molecular features. We aimed to explore differential genomic signatures in pNET subtypes and identify potential prognostic and therapeutic biomarkers.

Methods: We investigated genomic profiles of 57 LCNECs, 49 SCLCs, 18 TCs, and 24 ACs by sequencing tumor tissues with a 520-gene panel and explored the associations between genomic features and prognosis.

Results: Both LCNEC and SCLC displayed higher mutation rates for *TP53*, *PRKDC*, *SPTA1*, *NOTCH1*, *NOTCH2*, and *PTPRD* than TC and AC. Small cell lung carcinoma harbored more frequent co-alterations in *TP53-RB1*, alterations in *PIK3CA* and *SOX2*, and mutations in HIF-1, VEGF and Notch pathways. Large cell neuroendocrine carcinoma (12.7 mutations/Mb) and SCLC (11.9 mutations/Mb) showed higher tumor mutational burdens than TC (2.4 mutations/Mb) and AC (7.1 mutations/Mb). 26.3% of LCNECs and 20.8% of ACs harbored alterations in classical non-small cell lung cancer driver genes. The presence of alterations in the homologous recombination pathway predicted longer progression-free survival in advanced LCNEC patients with systemic therapy (P = .005) and longer overall survival (OS) in SCLC patients with resection (P = .011). The presence of alterations in VEGF (P = .048) and estrogen (P = .018) signaling pathways both correlated with better OS in patients with resected SCLC.

Conclusion: We performed a comprehensive genomic investigation on 4 pNET subtypes in the Chinese population. Our data revealed distinctive genomic signatures in subtypes and provided new insights into the prognostic and therapeutic stratification of pNETs.

Key words: pulmonary neuroendocrine tumor; next-generation sequencing; targetable driver alteration; homologous recombination; prognosis.

Implications for Practice

The study performed a comprehensive genomic investigation on the 4 histological subtypes of pulmonary neuroendocrine tumor in the Chinese population. We revealed distinctive genomic signatures in subtypes and identified potential prognostic biomarkers. Our study indicates that genomic profiling may complement histological evaluation to provide better prognostic and therapeutic stratification of pulmonary neuroendocrine tumor, which would help clinical management.

Introduction

Neuroendocrine tumors (NETs) consist of malignancies arising from neuroendocrine cells throughout various organs. The gastrointestinal tract and lung are the most frequently involved sites.[1] Pulmonary NETs (pNETs) account for approximately 25% of primary lung neoplasms.[2] According to the 2021 WHO classification, pNETs can be classified into 3 subtypes: preinvasive lesions (also known as diffuse idiopathic pulmonary neuroendocrine cell hyperplasia), neuroendocrine tumors composed of typical (TC) and atypical (AC) carcinoids, and neuroendocrine carcinomas inclusive of large cell neuroendocrine carcinoma (LCNEC) and small cell lung carcinoma (SCLC).[3,4] Small cell lung carcinoma is the most common pNET, representing ~20% of primary lung neoplasms, followed by LCNEC (3%), TC (2%), and AC (0.2%).[2]

Currently, surgery remains the only curative option for patients with carcinoid tumors,[5] whereas most patients with LCNEC or SCLC are often found metastasis or locally advanced disease at the time of diagnosis. For patients who cannot undergo surgery, chemotherapy or concurrent radiochemotherapy is the standard of care. The 2015 WHO classification differentiated LCNEC and large cell carcinoma due to the diverse heterogeneity observed between the 2 in terms of cytological features and biological and clinical responses to treatments. Large cell neuroendocrine carcinoma, along with SCLC, was reclassified under pNETs. However, some studies have revealed heterogeneity in the molecular features between LCNEC and SCLC. Rekhtman et al classified LCNEC into an SCLC-like subset, characterized by *TP53/RB1* co-mutation/loss, and an NSCLC-like subset, characterized by the lack of *TP53/RB1* co-alteration but the presence of NSCLC-type mutations.[6] In recent years, sequencing analyses conducted on pNETs have highlighted distinct genomic characteristics for different histological subtypes.[6-10] However, parallel comparison across all pNET subtypes with a single sequencing panel is limited, especially in eastern Asian populations.

Although it has been suggested that advanced LCNEC could be treated with SCLC-based regimens,[11,12] some phase II studies indicated an inferior response rate and prognosis to SCLC-based regimens (irinotecan-cisplatin or cisplatin-etoposide) in LCNEC patients compared with that in SCLC patients.[13,14] More recently, researchers demonstrated the prognostic role of genomic subtyping: NSCLC-like LCNEC tumors treated with NSCLC-type chemotherapy showed a more favorable prognosis than those treated with SCLC-type chemotherapy, while the survival outcomes in patients with SCLC-like LCNEC tumors were inconclusive for different chemotherapies.[7,15] There is still a lack of consensus regarding the optimal management of unresectable LCNEC and carcinoid tumors[5,16] due to their rarity and heterogeneity. Moreover, few studies on pNET have reported biomarkers for stratifying patients who are more likely to benefit from the current regimen.

In this multicenter study, we investigated the genomic characteristics of LCNEC, SCLC, and carcinoid tumors with targeted next-generation sequencing (NGS) to further compare the genomic landscapes among different histological subtypes of pNET and to explore genomic prognostic and actionable biomarkers for pNET in this cohort.

Materials and Methods

Patients' Information

Chinese patients diagnosed with pNET in 8 participating hospitals (Hunan Cancer Hospital/the Affiliated Cancer Hospital of Xiangya Medical School, Xiangya Hospital, Sun Yat-Sen University Cancer Center, Xinqiao Hospital, The Affiliated Cancer Hospital of Guangxi Medical University, Fujian Cancer Hospital, Cancer Hospital of Shantou University Medical College, Union Hospital Affiliated with Tongji Medical College of Huazhong University of Science and Technology) between June 2017 and December 2019 were enrolled. A comparable number of participants with each subtype were included. Neuroendocrine tumors were diagnosed according to the 2015 WHO histological classification of lung tumors.[17] All the tumors were reviewed by 2 independent pathologists to confirm the histological diagnosis. Pathological or clinical staging was based on the 7th edition of the American Joint Committee on Cancer.[18] Patients with unclear tumor stage, mixed histology samples, or a diagnosis of other malignant tumors within the previous 5 years were excluded from the study, resulting in a total of 148 patients eventually included. Tumor response assessment was based on Response Evaluation Criteria in Solid Tumors version 1.1.[19] Medical records were retrieved to extract data such as diagnostic information, treatment procedure, and survival. The study protocol was approved by the institutional review board (IRB) of Hunan Cancer Hospital. Due to the retrospective nature of the study, the requirement for informed consent was exempted by the IRB.

DNA Isolation and Capture-Based Targeted DNA Sequencing

Tissue DNA was extracted from formalin-fixed, paraffin-embedded (FFPE) tumor tissues using a QIAamp DNA FFPE tissue kit (Qiagen, Hilden, Germany). A minimum of 50 ng of DNA was used for NGS library preparation. Isolated DNA was sheared using Covaris M220 (Covaris, MA) and then subjected to end repair, phosphorylation, and adapter ligation. Fragments between 200 and 400 bp in size from the sheared genomic DNA were selected, purified using beads (Beckman Coulter, CA) and hybridized with capture probes of a panel consisting of 520 cancer-related genes spanning 1.64 Mb of the human genome (Burning Rock Biotech, Guangzhou, China). The quality and size of the library were assessed using a Qubit 2.0 fluorometer with the dsDNA high-sensitivity

assay kit (Life Technologies, Carlsbad, CA). Indexed samples were sequenced on Nextseq500 (Illumina, Inc.) with paired-end reads and a mean sequencing depth of 1698×.

Next-Generation Sequencing Data Analysis

Sequencing data in FASTQ format were mapped to the reference human genome (hg19) using Burrows-Wheeler Aligner v.0.7.10.[20] Local alignment optimization, duplication marking, and variant calling were performed using the Genome Analysis Tool Kit v.3.2[21] and VarScan v.2.4.3.[22] Variants were filtered using the VarScan fpfilter pipeline, and loci with a depth <100 were filtered out. Variants with population frequencies over 0.1% in the ExAC, 1000 Genomes, dbSNP, or ESP6500SI-V2 databases were grouped as single-nucleotide polymorphisms and excluded from further analysis. The remaining variants were annotated with ANNOVAR (2016-02-01 release)[23] and SnpEff v.3.6.[24] Analysis of DNA translocation was performed using Factera v.1.4.3.[25] The copy number variation (CNV) was estimated with an in-house algorithm based on the sequencing depth as described previously.[26] Copy number variation is called if the coverage data of the gene region were quantitatively and statistically significant from its reference control. The limit of detection for copy number variations is 1.5 for copy number deletion and 2.64 for copy number amplifications. Tumor mutational burden (TMB) per patient was computed as a ratio between the total number of nonsynonymous mutations detected with the coding region size of the panel using the formula below. Copy number variations, fusions, large genomic rearrangements, and mutations occurring on the kinase domains on *EGFR* and *ALK* were excluded from the mutation count.

$$TMB = \frac{\text{mutation count (except for copy number variations and fusion)}}{\text{total size of coding region counted}}$$

Pathway Analyses

Pathway analyses were based on all of the mutated genes identified. First, enrichment analyses were performed using the Database for Annotation, Visualization, and Integrated Discovery based on all metabolic and non-metabolic pathways in the Kyoto Encyclopedia of Genes and Genomes (KEGG). Pathways with Benjamini *P*-value <.05 were selected as candidates for subsequent comparisons on differential mutation prevalence of pathways in histological subtypes (Supplementary Table S1). A pathway was defined as mutated if an alteration was identified in any of the genes in the pathway. Pathways including the *TP53/RB1* gene were excluded for comparison due to the higher mutation rates in the 2 genes.

Statistical Analysis

Statistical analysis was performed using R version 3.3.3 software. Differences in the groups were calculated and presented using Fisher's exact test, paired 2-tailed Student's *t*-test, Wilcoxon test or analysis of variance as appropriate. Kaplan-Meier analysis was used to estimate survival, and a log-rank test was used to determine the differences in the multiple survival metrics between groups. *P*-values of <.05 were considered statistically significant.

Results

Distinctive Clinicopathological Characteristics, Treatments, and Prognosis in pNET Subtypes

We performed a comprehensive genomic profiling spanning 4 subtypes of pNET in the Chinese population. Among the 148 pNETs, 57 were LCNECs, 49 were SCLCs, and 42 were carcinoid tumors with 18 TCs and 24 ACs. The cohort had 26 (17.6%) females and 122 (82.4%) males and a median age of 59 years. The epidemiological characteristics of patients with different histological subtypes are summarized in Table 1, which illustrates that compared with SCLC and LCNEC patients, carcinoid patients were significantly younger and lacked a strong association with smoking, in line with a previous report.[17] More LCNEC and SCLC patients were diagnosed with stage IV disease (~50%), while more carcinoid patients presented early-stage disease (*P* < .0001).

The majority of SCLC (34/49, 69.4%) and LCNEC (36/57, 63.2%) patients had unresectable advanced diseases and only received systemic treatment, whereas 62.5% (15/24) and 100% of patients with ACs and TCs underwent resection, respectively. Of the 79 patients with advanced disease, 28 SCLC (82.4%), 12 LCNEC (33.3%), and 5 AC (55.6%) patients received a platinum-etoposide regimen as first-line treatment, while the remaining 25 were treated with other chemotherapy, targeted therapy, or immunotherapy. Detailed clinical outcomes for the 79 patients after systemic treatment are presented in Table 1 and demonstrate an overall response rate of 71.1% (37/52) and a disease control rate of 96.1% (50/52) in the 52 response evaluable patients after excluding those without the best response evaluation (*n* = 27, 34.2%). Total 19 LCNEC, 25 SCLC, and 9 AC patients receiving systemic treatment were assessed for progression-free survival (PFS), among whom 17 LCNEC, 24 SCLC, and 8 AC patients also had overall survival (OS) information. For early-stage patients who underwent surgery, 14 SCLCs, 17 LCNECs, and 31 carcinoids had both disease-free survival (DFS) and OS data (Supplementary Fig. S1). Different subtypes showed comparable PFS and OS after systemic treatment (Supplementary Fig. S1A and B). In patients with tumors resected, the carcinoid subtype displayed longer DFS and OS than LCNEC and SCLC (Supplementary Fig. S1C and D).

Genomic Characterization Revealed Differential Profiles Among pNET Subtypes

A total of 2456 somatic alterations in 414 genes were identified from 140 pNETs. Six TCs and 2 ACs had no mutations detected from this panel. The most frequently mutated genes were *TP53* (75%) and *RB1* (43%; Supplementary Fig. S2). Mutational comparisons (Fig. 1A) revealed that both LCNEC (89.3%) and SCLC (98.0%) displayed a higher rate of *TP53* mutations than carcinoid tumors (28.6%; *P* < .0001 and *P* < .0001). SCLC patients (85.7%) harbored more *RB1* alterations than LCNEC (26.3%, *P* < .0001) and carcinoid patients (16.7%, *P* < .0001). *TP53* and *RB1* co-alteration was present in 83.7% of SCLCs, but only in 26.3% (*P* < .0001) of LCNECs and 14.3% (*P* < .0001) of carcinoids. Moreover, *PRKDC*, *SPTA1*, *NOTCH1*, *NOTCH2*, and *PTPRD* were mutated more frequently in SCLCs and LCNECs than in carcinoids (Fig. 1A). The mutation rates of *PIK3CA* and *SOX2* were higher in SCLCs than in LCNECs (*P* = .011, *P* = .019) and carcinoids (*P* = .034, *P* = .018). Next, we performed pathway analyses

Table 1. Clinicopathological and epidemiological characteristics of patients.

Characteristic	Total (*n* = 148)	AC (*n* = 24)	TC (*n* = 18)	LCNEC (*n* = 57)	SCLC (*n* = 49)	*P*-value
Age, years						<.0001
Median[Q1, Q3]	59[51.5-65]	52[47.5, 63.5]	50[41.25, 53]	62[56, 67]	60[54, 65.5]	
Gender, no. (%)						<.00001
Female	26(17.6%)	10(41.7%)	8(44.4%)	2(3.5%)	6(12.2%)	
Male	122(82.4%)	14(58.3%)	10(55.6%)	55(96.5%)	43(87.8%)	
Smoking, no. (%)						<.001
No	34(23%)	9(37.5%)	11(61.1%)	7(12.3%)	7(14.3%)	
Yes	102(68.9%)	14(58.3%)	6(33.3%)	43(75.4%)	39(79.6%)	
NA	12(8.1%)	1(4.2%)	1(5.6%)	7(12.3%)	3(6.1%)	
Stage, no. (%)						<.0001
I	31(20.9%)	8(33.3%)	11(61.1%)	7(12.3%)	5(10.2%)	
II	12(8.1%)	2(8.3%)	1(5.6%)	6(10.5%)	3(6.1%)	
III	32(21.6%)	4(16.7%)	1(5.6%)	15(26.3%)	12(24.5%)	
IV	57(38.5%)	8(33.3%)	0(0%)	27(47.4%)	22(44.9%)	
NA	16(10.8%)	2(8.3%)	5(27.8%)	2(3.5%)	7(14.3%)	
Treatment, no. (%)						<.00001
Systemic	79(53.4%)	9(37.5%)	0(0%)	36(63.2%)	34(69.4%)	
Surgery	69(46.6%)	15(62.5%)	18(100%)	21(36.8%)	15(30.6%)	
[a]Best response, no. (%)						<.01
PD	2(2.5%)	1(11.1%)	—	0(0%)	1(2.9%)	
PR	37(46.8%)	2(22.2%)	—	13(36.1%)	22(64.7%)	
SD	13(16.5%)	3(33.3%)	—	5(13.9%)	5(14.7%)	
NA	27(34.2%)	3(33.3%)	—	18(50.0%)	6(17.6%)	
Systemic regimen, no. (%)						<.001
EP	34(43%)	4(44.4%)	—	10(27.8%)	20(58.8%)	
EC	11(13.9%)	1(11.1%)	—	2(5.6%)	8(23.5%)	
IO	2(2.5%)	0(0%)	—	2(5.6%)	0(0%)	
EGFR-TKI	2(2.5%)	0(0%)	—	2(5.6%)	0(0%)	
Other chemotherapy	11(13.9%)	2(22.2%)	—	8(22.2%)	1(2.9%)	
NA	19(24.1%)	2(22.2%)	—	12(33.3%)	5(14.7%)	

TC: typical carcinoid; AC: atypical carcinoid; LCNEC: large cell neuroendocrine carcinoma; SCLC: small cell lung cancer; CR: complete response; PR: partial response; SD: stable disease; PD: progression disease; EP: etoposide + cisplatin; EC: etoposide+ carboplatin; IO: immunotherapy;
[a]Best response evaluated upon systemic treatment for unresectable tumors.

based on the mutated genes identified from each subtype and explored functionally different pathways. The mutational prevalence for genes in 25 enriched KEGG pathways was compared among different subtypes (Supplementary Table S1). The results revealed that mutation rates in most pathways were significantly higher in SCLCs and LCNECs than in carcinoids (Fig. 1B), which is likely attributed to the low mutation frequency in carcinoids. In addition, SCLCs displayed higher mutation rates in the HIF-1 (*P* = .011), VEGF (*P* = .029), and Notch (*P* < .01) signaling pathways than LCNECs.

For CNV analysis, we observed that TCs (median count: 0) had significantly fewer CNV events than SCLCs (median count: 4.0, *P* < .0001), LCNECs (median count: 2.0, *P* < .0001), and ACs (median count: 0.5, *P* < .001), whereas ACs exhibited lower CNV counts than SCLCs (*P* = .018; Fig. 1C). Tumor mutational burden analysis revealed a comparable TMB status of LCNECs (12.7 mutations/Mb) and SCLCs (11.9 mutations/Mb) but a significantly lower TMB in both TCs (2.4 mutations/Mb, *P* < .0001, *P* < .01) and ACs (7.1 mutations/Mb, *P* < .0001, *P* < .01; Fig. 1D).

We further classified 57 LCNECs into subsets according to Rekhtman et al[6]: 15 LCNECs with concomitant *TP53/RB1* alteration were defined as SCLC-like; 25 LCNECs lacking *RB1* alteration but harboring alterations in *STK11*, *ERBB2*, *MET*, *KRAS*, *KEAP1*, or *EGFR* were defined as NSCLC like; and the remaining 17 LCNECs with neither *RB1* nor NSCLC-type mutations were grouped into a third subset (others). In addition to *RB1* alteration, *PTEN* alteration was exclusive to the SCLC-like subset (6/15) (Supplementary Fig. S3A), whereas alterations in *KEAP*, *STK11*, and *KRAS* were only present in the NSCLC-like subset. Three subsets of LCNEC possessed comparable TMB status (Supplementary Fig. S3B). An elevated CNV count was observed in the SCLC-like subset compared with the third subset (others) (*P* = .02, Supplementary Fig. S3C).

Alterations in Non-Small-Cell Lung Cancer Targetable Driver Genes Identified in LCNEC and Carcinoid Tumors

Given the lack of consensus on the optimal treatment for unresectable or metastatic LCNEC and carcinoid tumors,
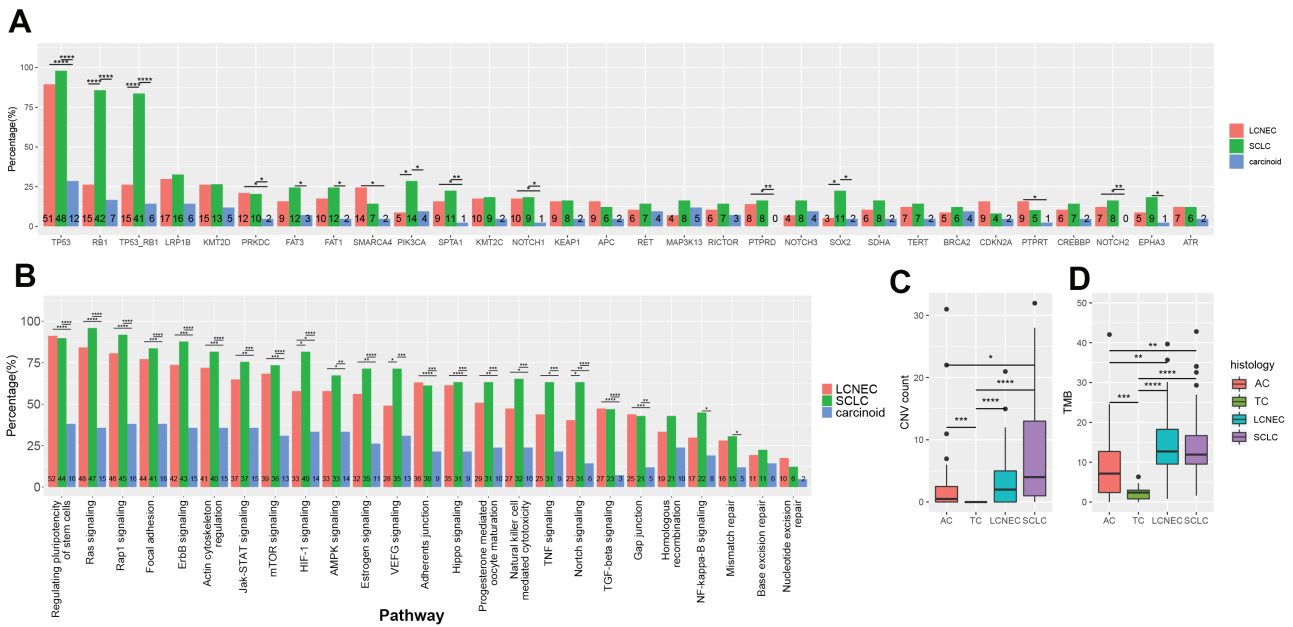
Figure 1. The comparison of genomic features in different histological sub-cohorts. (**A**) gene mutated frequency; (**B**) pathway mutated frequency, only pathways with mutations >10 are listed and pathways including *TP53* or *RB1* are excluded; (**C**) copy number variation (CNV); (**D**) tumor mutational burden (TMB); LCNEC (*n* = 57), SCLC (*n* = 49), carcinoids (*n* = 36); (*P < .05, **P < .01, ***P < .001, ****P < .0001).

we investigated the alterations in non-small-cell lung cancer (NSCLC) targetable driver genes that were present in our LCNEC and carcinoid sub-cohorts. A total of 24 alterations in classic NSCLC driver genes (9 missense and 15 amplifications) were observed in 26.3% of LCNECs (*n* = 15) and 20.8% of ACs (*n* = 5) (Table 2) but none was found in TCs. In LCNEC, 44% of NSCLC-like subset (*n* = 11) harbored driver alterations versus 26.7% in SCLC-like subset (*n* = 4; *P* = .2789). Interestingly, the hotspot actionable mutation *EGFR* p.L858R was detected in 3 NSCLC-like and one SCLC-like LCNEC patients, 3 of whom also harbored an additional driver alteration. One NSCLC-like LCNEC patient harbored the *KRAS* hotspot mutation p.G12C. The remaining driver mutations were less common, including *KRAS* p.Q61L (*n* = 1), p.G13C (*n* = 1), *BRAF* p.D594G (*n* = 1), and *EGFR* p.E709K (*n* = 1). Amplifications in the driver genes were observed in 6 NSCLC-like (3 *EGFR*, 2 *KRAS*, and 1 *ERBB2*) and 2 SCLC-like LCNEC (2 *MET*) patients. Of note, the driver alterations found in the 5 AC patients were all amplifications, including 4 with *ERBB2* amplification and 2 with *KRAS* amplification. As expected, none of the 17 LCNEC patients with genomic subtyping of others harbored classical NSCLC driver alterations; instead, 3 of them carried alterations in non-NSCLC driver genes (one with *HRAS* p.Q61L, one with *PIK3CA* p.H1047R, and one with amplifications in *PDGFRA* and *KIT*).

Notably, 2 of the 4 patients harboring *EGFR* p.L858R underwent EGFR TKI treatment. Case L051, who was diagnosed with stage IV LCNEC with SCLC-like genomic subtyping, received the first-line treatment of gefitinib and achieved partial response (PR) with a PFS of 15 months. The patient also harbored a concurrent *EGFR* p.E709K. Case L043, who had stage IVb LCNEC with NSCLC-like genomic subtyping, was administered pemetrexed for one cycle and switched to gefitinib upon detection of *EGFR* p.L858R. The patient achieved PR 2 months after gefitinib initiation lasting for 8 months and had an OS of 12 months.

Alterations in Homologous Recombination (HR), VEGF, or Estrogen Signaling Pathway Predicted Better Prognosis in pNET

We further explored potential prognostic biomarkers associated with patients' clinical outcomes in the different pNET subtypes. In advanced LCNEC patients treated systemically, alterations in the HR signaling pathway correlated with longer PFS (*n* = 19, 14 months vs 6 months, *P* = .005, Fig. 2A) but not with OS (*n* = 17, *P* = .294; Fig. 2B). In the subset of LCNEC patients treated with platinum-based chemotherapy (*n* = 11), those harboring HR mutations (*n* = 3) also showed a trend of longer PFS (*P* = .06, Supplementary Fig. S4A). However, the same trend was not observed in SCLC patients treated with platinum-based chemotherapy (Supplementary Fig. S4C). In the 14 SCLC patients underwent surgery, alterations in the HR pathway also correlated with longer OS (not reached vs 16 months, *P* = .011), but not with DFS (*P* = .338; Fig. 2C and D).

Next, the association between alterations in homologous recombination (HR), VEGF, or estrogen signaling pathway and survival outcomes were explored in the 14 surgically treated SCLC patients. It is well known that surgically resected disease implies a lower stage and, by extension, longer survival. In order to explore whether tumor stage impacts on the survival outcomes, the resected SCLC patients were stratified for tumor stage. We found that there was no difference of DFS (Supplementary Fig. S5A) and OS (Supplementary Fig. S5B) among patients with stages I, II, and III disease, which indicated that tumor stage was not significantly correlated with survival outcomes (DFS/OS) in the present work. Moreover, alterations in the VEGF signaling pathway were associated with better DFS (24 months vs 9 months, *P* = .051) and OS (NR vs 16 months, *P* = .048) in the 14 surgically treated SCLC patients (Fig. 3A and B). In the same set of patients, we also observed a significant association between estrogen signaling pathway alteration and longer OS (NR vs 16 months, *P* = .018), whereas the association was not significant

Table 2. Classic NSCLC driver alterations in LCNEC and carcinoid.

Patient ID	Gene	Alteration	Therapeutic evidence[a]	Subtype
L027	*EGFR*	p.L858R	1	NSCLC-like LCNEC
L014	*EGFR*	p.L858R	1	NSCLC-like LCNEC
	KRAS	p.Q61L	4	
L043	*EGFR*	p.L858R	1	NSCLC-like LCNEC
	EGFR	cn_amp	—	
L017	*ERBB2*	cn_amp	2	NSCLC-like LCNEC
L041	*KRAS*	p.G12C	3A	NSCLC-like LCNEC
L003	*KRAS*	p.G13C	4	NSCLC-like LCNEC
L052	*EGFR*	cn_amp	—	NSCLC-like LCNEC
L054	*EGFR*	cn_amp	—	NSCLC-like LCNEC
L002	*KRAS*	cn_amp	—	NSCLC-like LCNEC
L016	*KRAS*	cn_amp	—	NSCLC-like LCNEC
L031	*BRAF*	p.D594G	—	NSCLC-like LCNEC
L051	*EGFR*	p.L858R	1	SCLC-like LCNEC
	EGFR	p.E709K	—	
L013	*MET*	cn_amp	2	SCLC-like LCNEC
L035	*MET*	cn_amp	2	SCLC-like LCNEC
L012	*EGFR*	cn_amp	4	SCLC-like LCNEC
C018	*ERBB2*	cn_amp	2	AC
C019	*ERBB2*	cn_amp	2	AC
C020	*ERBB2*	cn_amp	2	AC
C021	*ERBB2*	cn_amp	2	AC
	KRAS	cn_amp	—	
C024	*KRAS*	cn_amp	—	AC

[a]Levels of evidence are adopted from OncoKB database: (1) FDA-recognized biomarker predictive of response to an FDA-approved drug in this indication; (2) standard care biomarker recommended by the NCCN or other expert panels predictive of response to an FDA-approved drug in this indication; (3A) compelling clinical evidence supports the biomarker as being predictive of response to a drug in this indication; (4) compelling biology evidence supports the biomarker as being predictive of response to a drug. AC: atypical carcinoid; LCNEC: large cell neuroendocrine carcinoma; SCLC: small cell lung cancer; NSCLC: non-small cell lung cancer.

with DFS (*P* = .094; Fig. 3C and D). Due to the limited cases of resected SCLC patients, Cox regression analyses were not subsequently performed to investigate whether the presence of alterations in VEGF, HR, and estrogen signaling pathways were independent factors associated with survival outcomes.

By contrast, mutations in *TERT* or *KEAP1* appeared to correlate with an unfavorable prognosis. In advanced SCLC patients with systemic treatment, those with *TERT* or *KEAP1* mutation show a trend of shorter PFS (*n* = 25, 4 months vs 8 months, *P* = .072, Supplementary Fig. S6A) than those without *TERT* and *KEAP1* mutations, but no difference was found in OS between these 2 subgroups (*n* = 24, *P* = .967, Supplementary Fig. S6B).

We did not identify any prognosis-correlated alterations in carcinoids probably owing to its low mutational frequency and a limited number of patients. Patients with NSCLC-like LCNECs had inferior PFS (6.5 months vs 13.0 months, *P* = .045) but similar OS compared with those with SCLC-like LCNECs following systemic treatment (Supplementary Fig. S7A and B). In patients undergoing surgery, the 2 subsets of LCNECs showed similar DFS and OS, and both were also comparable with SCLCs (Supplementary Fig. S7C and D).

Discussion

Previous studies have reported frequent inactivating mutations in *TP53* and *RB1*, copy number amplification of members in the MYC family, and genetic alterations in Notch family members and PI3K/AKT/mTOR pathway in SCLC,[7,8,27] and carcinoids are characterized by frequent alterations in chromatin remodeling genes.[28] The genomic profile of LCNEC revealed biological heterogeneity, comprising distinct subsets with signatures of SCLC, NSCLC, and rarely, carcinoids.[6,28] Herein, we present a parallel study comparing the genomic landscapes of all subtypes of pNET in Chinese population. In general, the mutational profile in our cohort resembles that of the Western population with subtle differences. *SOX2*, an MYC family member, was identified as a frequently amplified gene in SCLC (27%-31%) [7,29] but a less common alteration in LCNEC (~3%).[7] Concordantly, we observed that 22.4% of SCLCs harbored the *SOX2* amplification, which is significantly higher than the proportion in LCNECs (5%) and carcinoid tumors (5.6%; Fig. 1A). We also revealed enriched mutations in *PRKDC*, *SPTA1*, and *PTPRD* in SCLC and LCNEC subtypes (Fig. 1A). The *PRKDC/SPTA1/PTPRD* mutations have seldom been reported in Caucasian SCLC/LCNEC studies; however, a recent study of SCLC in the Chinese population showed a *SPTA1* mutation frequency of 11.5%,[29] together with our observation, suggesting that some of the gene mutations are ethnicity-dependent signatures.

We found 26.3% (15/57) of LCNEC patients who underwent NGS analysis harbored *RB1* alterations and all *RB1*-mutant LCNEC patients carried concurrent *TP53* alterations. To the best of our knowledge, 2 and 4 previous studies have documented the genomic profiling of LCNEC in Chinese[15,30] and Western population,[6,7,15,31] respectively. The *RB1*-mutatnt frequency in Chinese LCNEC patients has been reported to be 35.7% and 32.1%, which was 29.0%, 35.6%, 41.7%, and 46.8% in Western LCNEC patients as previously reported. We found that there was no significant difference of the percentage of *RB1* alterations in the present work with that reported in previous studies.[6,7,15,30,31] The question of whether the *RB1*-mutant frequency in Chinese LCNEC patients is significantly different from that in Western patients is needed to be investigated in a large cohort study encompassing Chinese and Western LCNEC patients.

We observed 26.3% of the 57 LCNECs harboring alterations in classical NSCLC driver genes (Table 2), including missense mutations in *EGFR*, *KRAS*, and *BRAF* (*n* = 9) and amplifications in *EGFR*, *KRAS*, *ERBB2*, and *MET* (*n* = 9). Comparably, Miyosh et al reported activating alterations in targetable RTK genes in 23% of 78 LCNECs.[9] On the other
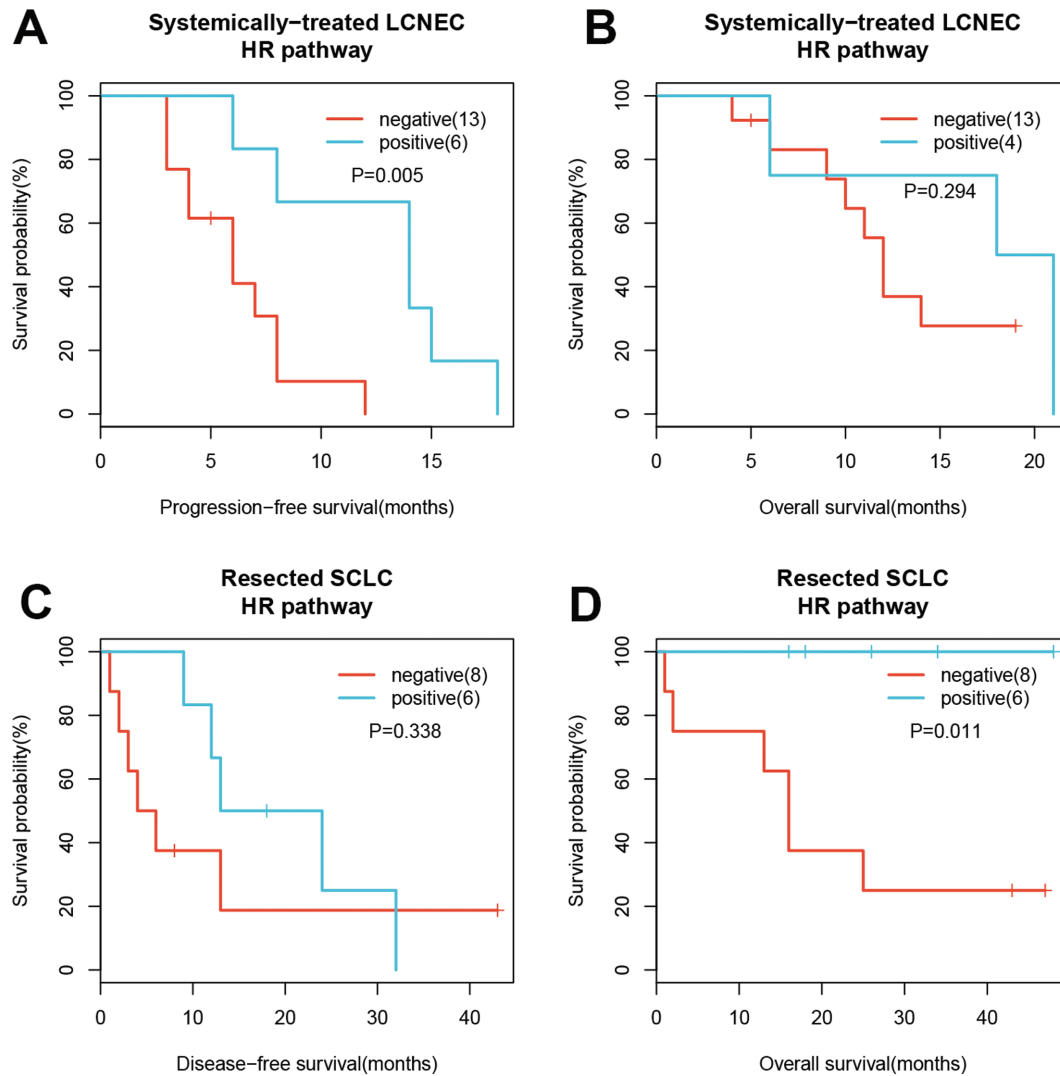
Figure 2. The association of alteration in homologous recombination (HR) signaling pathway with prognosis in LCNEC and SCLC. (**A**, **B**) Progression-free survival and OS in systemically treated patients with LCNEC; (**C**, **D**) DFS and OS in patients with resected SCLC.

hand, only amplifications in driver genes (*ERBB2* and *KRAS*) were identified in ACs (Table 2). Overall, our results highlight the potential of using targeted therapy in LCNEC and AC patients.

Of note, we identified an actionable *EGFR* p.L858R in 4 LCNECs (7%). Zhuo et al recently reported actionable *EGFR* p.L858R, *EGFR* T790M, and *ALK* fusion in 4, 1, and 1 Chinese LCNEC patients, respectively (*n* = 44).[15] Miyosh et al also described an actionable *EGFR* 19del in a Japanese LCNEC patient.[9] However, these classical actionable mutations have rarely been reported in Caucasian patients, implying the potential distinctive etiology of East Asian patients from that of Westerners.[15] 2 of the patients harboring L858R in our study received gefitinib and achieved PR, with one achieving a relatively long PFS of 15 months. Similarly, several studies reported responses in *EGFR* L858R/19del-positive LCNEC patients treated with EGFR-TKIs.[15,32,33] Therefore, *EGFR*-TKIs should serve as a therapeutic option for *EGFR*-mutant patients with advanced LCNEC.

In the present work, 42.9% of (18/42) carcinoids patients harbored alterations in genes implicated in covalent histone modification/chromatin remodeling, including *MEN1* (16.7%, 7/42), *ARID1A* (7.1%, 3/42), *KMT2A* (4.8%,

2/42), *KMT2C* (4.8%, 2/42), *KMT2D* (11.9%, 5/42), and *SMARCA4* (4.8%, 2/42), reproducing the results from the previous studies performed in Western population.[34,35] Although *TP53* and *RB1* alterations have been reported to be rare events in carcinoids in Western population,[34,35] these 2 genes were frequently altered in our work, occurring in 28.6% (12/42) and 16.7% (7/42) of carcinoid patients, respectively. Low-density lipoprotein receptor related protein 1B (*LRP1B*) alterations and *ERBB2* amplification have not been documented in lung carcinoids, but these 2 genes were recurrently altered (25.0% and 16.7%) in atypical carcinoid in the present work. These findings suggest that Chinese lung carcinoid patients have unique molecular characteristics.

Few studies have reported the prognostic value of genomic alterations in pNET. Simbolo et al reported *TERT* amplification as a predictor of poor prognosis in pNETs regardless of histology.[10] Similarly, we revealed a trend that alterations in *TERT* or *KEAP* associated with shorter PFS in SCLC patients with systemic treatment (Supplementary Fig. S5A). Increased *TERT* mRNA expression and mutation of the *TERT* promoter have also been reported to correlate with poor prognosis in NSCLC.[36,37] On the other hand, Keap1-Nrf2 pathway activation plays an important role in acquiring resistance
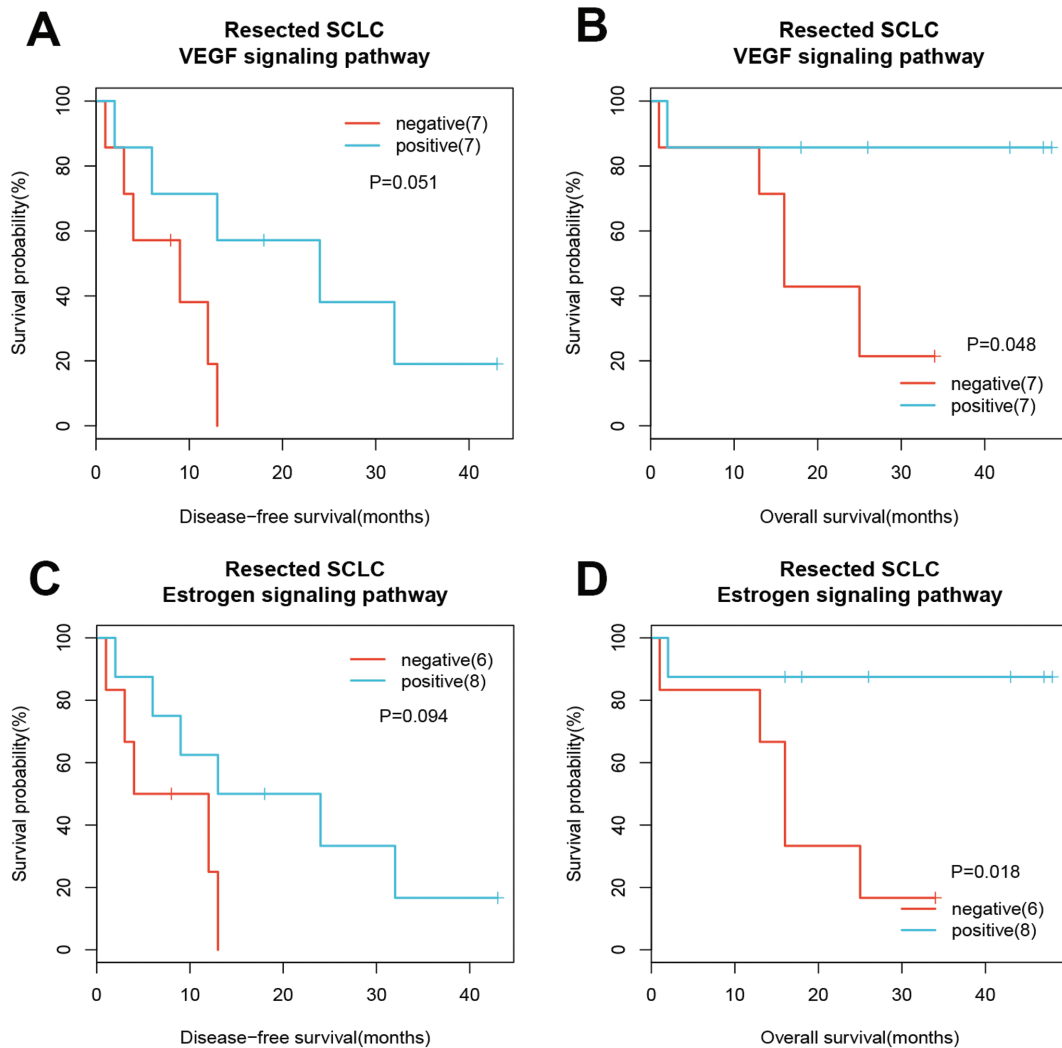
Figure 3. The associations of alterations in VEGF and estrogen signaling pathways with prognosis in patients with resected SCLC.

to chemotherapy, including platinum-based treatments, in NSCLC.[38,39] *KEAP1* mutations were associated with a worse prognosis and shorter postoperative DFS in NSCLC.[40]

Our study is the first to identify mutations in the HR and estrogen signaling pathways as predictive markers in SCLC/LCNEC. Homologous recombination is one of the major repair pathways for DNA double-strand breaks. Emerging evidence suggests that tumors with mutations in the HR process are sensitive to drugs targeting the DNA repair pathway, such as platinum agents and PARP inhibitors.[41–44] In our study, mutations in the HR pathway predicted better prognosis in systemically treated advanced LCNEC patients and surgically treated SCLC patients (Fig. 2). Of note, most LCNEC patients in our cohort who received systemic therapy were treated with a platinum-based regimen, which is in line with the proposed association of the presence of HR mutations and favorable survival to the platinum-based treatment in LCNEC patients. Moreover, a subset of SCLC patients who underwent surgery also received adjuvant platinum therapy. This might explain our observation that patients with HR-mutant resected SCLC had longer DFS and OS. The homologous recombination deficiency (HRD) score integrates DNA-based measures of genomic instability and has been approved as a marker to refine treatment selection for PARP inhibitor in advanced ovarian

cancer.[45] It would be interesting to assess the association between HR mutation and HRD score and to explore if HRD score can also serve as a predictor in SCLC/LCNEC.

The expression of estrogen receptor alpha (ERα) and estrogen receptor beta (ERβ) as prognostic markers has been extensively studied in NSCLC. However, the results have been inconclusive and even contradictory.[46-48] A study performed in 126 patients with resected pNETs revealed a beneficial survival in the subgroup of male SCLC patients with positive ERβ expression in tumors ($n = 15$, $P = .008$).[49] Our study demonstrated that mutations in the estrogen signaling pathway may also predict longer OS in patients with resected SCLC. However, the prognostic role of genes in the estrogen pathway in SCLC and other pNETs, as well as the underlying mechanisms, merit further investigation.

We also demonstrated that mutations in the VEGF pathway may predict better prognosis in SCLC patients (Fig. 3A and B). Genes in the VEGF pathway regulate the central pathophysiology of tumor angiogenesis.[50] Evidence suggests that angiogenesis in SCLC plays a fundamental role in tumor growth, invasiveness and metastases, and mediates resistance to chemotherapy.[51] The inactivation of the VEGF pathway (lower VEGF level) in SCLC has important prognostic implications, acting as a favorable prognostic factor in most

cases.[52–54] Our results in line with previous studies suggest the prognostic role of VEGF pathway mutation in SCLC.

Rekhtman et al demonstrated that *RB1* wild-type (NSCLC-like) LCNEC tumors treated with NSCLC-type chemotherapy (platinum+gemcitabine or taxanes) showed favorable prognosis than those treated with SCLC-type chemotherapy (platinum-etoposide); however, no difference was observed for prognosis in *RB1*-mutated (SCLC-like) LCNEC patients with different chemotherapy regimens.[7] Intriguingly, we observed an inferior median PFS in patients with NSCLC-like LCNECs compared with those with SCLC-like LCNECs (Supplementary Fig. S6A) following systemic treatment. Given that the majority of the systemically treated LCNEC patients in our study received a platinum-etoposide regimen, our results further indicate that LCNEC patients of NSCLC-like genomic subtyping may benefit less from SCLC-type chemotherapy, therefore should be treated with other regimens.

Our study encompassed a large cohort of Chinese pNET patients. However, due to the retrospective nature of this study, the follow-up was not available for a number of patients, resulting in a limited sample size with prognostic information for each subtype. In addition, there was heterogeneity in the treatments administered to patients, especially for the LCNEC subtype, which might weaken the strength of our discovery of prognostic markers. Therefore, well-designed prospective studies with larger cohorts are required to validate our findings. Last but not at least, large cohort study is needed to verify whether VEGF, HR, and estrogen signaling pathways are independent factors associated with survival outcomes in pNETs in the further work.

In conclusion, our comprehensive investigation of the genomic signature encompassed all subtypes of pNET in the Chinese population. Our data indicate that genomic profiling may complement histological evaluation to provide better prognostic and therapeutic stratification of pNETs, which would help clinical management.

Supplementary Material

Supplementary material is available at *The Oncologist* online.

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Conflict of Interest

Lin Wu: AstraZeneca, Roche, Bristol-Myers Squibb, MSD, Pfizer, Lilly, Boehringer Ingelheim, Merck, Innovent and Hengrui (C/A); Burning Rock Biotech (E). Lin Shao, Haiwei Du, Ting Hou, Zhiqiu Chen, and Jianxing Xiang are from Burning Rock Biotech (E). The other authors indicated no financial relationships.

(C/A) Consulting/advisory relationship; (RF) research funding; (E) employment; (ET) expert testimony; (H) honoraria received; (OI) ownership interests; (IP) intellectual property rights/inventor/patent holder; (SAB) scientific advisory board.

Author Contributions

Conception/design: L.W. Provision of study material or patients: Liming.C., Likun.C., G.L., B.Z., X.H., Y.L., S.Z. Collection and/or assembly of data: W.P., M.J., J.W., Z.C., X.P., F.X., J.L., T.H. Data analysis and interpretation: T.H. Data interpretation and pathogenic diagnosis review: J.L., C.L. Manuscript writing: J.X., L.S., H.D., W.P., L.W. Final approval of manuscript: all authors.

Data Availability

The data underlying this article will be shared on reasonable request to the corresponding author.

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