Original Article

Molecular features of gastroenteropancreatic neuroendocrine carcinoma: A comparative analysis with lung neuroendocrine carcinoma and digestive adenocarcinomas

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Abstract

Objective: There is an ongoing debate about whether the management of gastroenteropancreatic (GEP) neuroendocrine carcinoma (NEC) should follow the guidelines of small-cell lung cancer (SCLC). We aim to identify the genetic differences of GEPNEC and its counterpart.

Methods: We recruited GEPNEC patients as the main cohort, with lung NEC and digestive adenocarcinomas as comparative cohorts. All patients undergone next-generation sequencing (NGS). Different gene alterations were compared and analyzed between GEPNEC and lung NEC (LNEC), GEPNEC and adenocarcinoma to yield the remarkable genes.

Results: We recruited 257 patients, including 99 GEPNEC, 57 LNEC, and 101 digestive adenocarcinomas. Among the mutations, *KRAS*, *RB1*, *TERT*, *IL7R*, and *CTNNB1* were found to have different gene alterations between GEPNEC and LNEC samples. Specific genes for each site were revealed: gastric NEC (*TERT* amplification), colorectal NEC (*KRAS* mutation), and bile tract NEC (*ARID1A* mutation). The gene disparities between small-cell NEC (SCNEC) and large-cell NEC (LCNEC) were *KEAP1* and *CDH1*. Digestive adenocarcinoma was also compared with GEPNEC and suggested *RB1*, *APC*, and *KRAS* as significant genes. The *TP53*/*RB1* mutation pattern was associated with first-line effectiveness. Putative targetable genes and biomarkers in GEPNEC were identified in 22.2% of the patients, and they had longer progression-free survival (PFS) upon targetable treatment [12.5 months *vs.* 3.0 months, HR=0.40 (0.21−0.75), P=0.006].

Conclusions: This work demonstrated striking gene distinctions in GEPNEC compared with LNEC and adenocarcinoma and their clinical utility.

Keywords: Neuroendocrine carcinoma; gastroenteropancreatic; lung; genetic alterations; molecular markers

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Introduction

Poorly differentiated gastroenteropancreatic (GEP) neuroendocrine carcinoma (NEC), a more aggressive form of neuroendocrine neoplasms (NENs), is gaining much attention (1). The annual incidence rate is 0.54 per million people, but over two-thirds of GEPNEC is accompanied by disseminated disease, which always has a poor prognosis ranging from 5 to 14 months (metastatic disease). With very few studies on the carcinogenic mechanisms associated with GEPNEC, treatment options are scarce and mainly comply with small-cell lung cancer (SCLC) according to the morphological similarities (2). However, a comparative study of lung and extrapulmonary NEC using the Surveillance, Epidemiology, and End Results (SEER) database revealed tremendous clinical disparities (3).

Gene sequencing approaches have discovered the relationship among lung NECs (LNECs) [SCLC and lung large-cell neuroendocrine carcinoma (LLCNEC)]. Accumulating data support the use of genomic approaches to differentiate GEPNEC from local adenocarcinoma (3). However, the genomic profile of GEPNEC remains poorly investigated due to its rarity. Puccini *et al.* suggested that *TP53*, *RB1*, and *KRAS* were the most commonly altered genes (4). Venizelos *et al*. delineated the currently largest sample size molecular analyses of high-grade GEPNENs. This study showed a significant difference between NET G3 and NEC and substantial differences among different primary sites, confirming the results of the present study (5). Yachida *et al*. depicted the most comprehensive molecular landscape of high-grade GEP of unknown origins (6), demonstrating higher frequencies of *TP53*, *RB1*, and *KRAS* genomic alterations in NEC compared with local G3 NET (ATRX/DAXX mutations) (4,7,8). Other small-sample studies provided molecular features of GEPNEC of various anatomical sites and revealed *KRAS*, *BRAF*, and *PI3KCA* as targetable genes (7,9-22). However, these studies are predominantly based on data from Caucasian populations, and there is a lack of genomic information on GEPNEC in the Chinese population.

Current guidelines recommend treating GEPNEC following the steps for SCLC. However, emerging studies on specific sites, such as colorectal NEC (CRNEC) and pancreatic NEC (PNEC), suggest treatments that resemble local adenocarcinoma treatments, implying that other therapies may be utilized in these cases (16,18). Nevertheless, no direct comparisons have been made between GEPNEC and their lung counterparts or adenocarcinoma.

We aimed to elucidate the genetic characteristics of GEPNEC by comparing them with LNEC and local adenocarcinoma using targeted next-generation sequencing (NGS) and further identify the distinct landscapes of GEPNEC in relation to lung NEC (anatomical perspectives) and local adenocarcinoma (pathological perspectives). These findings may help pave the way for novel tailored therapies in future.

Materials and methods

Patients and samples

We retrospectively analyzed NEC patients treated at Peking University Cancer Hospital between January 1, 2016, and December 1, 2020. The main cohort, referred to as GEPNEC, can be separated into the following subgroups: esophagus, gastric, duodenum, colon, rectum, pancreas and biliary tract, and unknown primary NEC, including NEC originating from the liver, mesentery, and peritoneal cavity with suspected digestive system origins. The comparative cohort was comprised of lung NECs (including SCLC and LLCNEC) and digestive adenocarcinomas (primary sites included stomach, colorectum, bile tract, and pancreas). Prior to molecular examination, tumor enrichment formalin-fixed paraffin-embedded (FFPE) tumor samples were obtained by biopsies or harvesting tissue. The FFPE samples were reviewed, and the diagnoses were immunohistochemically (IHC) confirmed by two independent pathologists according to the 2019 5th edition of the World Health Organization Classification of Neuroendocrine Tumors (1). Any contradictions were judged by a third expert. Samples misdiagnosed or ambiguously diagnosed (e.g., NET G3), failed quality control, contained no more than 50% tumor cells, or lacked basic clinical information were excluded.

Inclusion criteria for the main cohort included: 1) Pathological diagnosis: it was confirmed as NEC by two

independent pathologists according to the 2010 World Health Organization Classification of Neuroendocrine Neoplasms and was judged by a third pathologist when necessary (10,11); 2) it had completed clinical information that could be obtained from the Hospital Information System; and 3) aged 18 years or older. Exclusion criteria included: 1) origins from neither digestive system nor lung; 2) no adequate histological specimens; or 3) no baseline clinical information. We obtained informed consent from all patients in accordance with the ethical guidelines of the Helsinki Declaration. This study protocol was approved by the Institutional Review Boards of Peking University Cancer Hospital (No. 2020YJZ92).

The patients had prospective follow-up visits every six weeks, with radiologic evaluations [based on Response Evaluation Criteria in Solid Tumors version 1.1 (RECIST 1.1)] and laboratory tests. The disease control rate (DCR) was calculated as stable disease (SD), and the objective response was summarized as complete remission (CR) or partial response (PR). Progression-free survival (PFS) was defined as the time from the initiation of each line of therapy to documented progressive disease (PD) or death due to any cause. Overall survival (OS) was defined as the time from the initiation of the therapy to death. If no endpoint was observed at the time of the analysis, the patients were censored at the time of their last follow-up.

NGS-based genomic profiling

DNA was extracted from FFPE specimens obtained from biopsy or surgery and whole blood control samples using QIAamp DNA FFPE Tissue Kit and DNeasy Blood and Tissue kit (Qiagen, Berlin, Germany). Potential artifacts from the FFPE samples were excluded by a strict quality control process. The DNA concentration was measured using a Qubit 3.0 fluorometer (ThermoFisher, Carlsbad, USA). Library construction was performed using 1–2 μg of DNA and a KAPA Hyper Prep kit (KAPA Biosystems, San Diego, USA). DNA libraries were then used to generate target-enriched amplicons using a Geneseeq Prime panel (233 cancer-related genes). Constructed libraries were sequenced using Hiseq 4000 NGS platforms (Illumina, San Diego, USA). The mean sequencing depth arrived at 1,100× and was qualified for the analysis. The experiment was performed in a centralized clinical testing center (Nanjing Geneseeq Technology Inc., Nanjing, China) following the protocol reviewed and approved by the Ethical Committee of Peking University Cancer Hospital.

Data processing

We applied rigorous quality control procedures before further analyses, following the protocols described in the Picard tool (http://broadinstitute.github.io/picard/). NGS data were aligned to the hg19 reference human genome using the Burrows-Wheeler Aligner (bwa-mem) (23) and then were processed using the Picard suite (https://broadinstitute.github.io/picard/) and the Genome Analysis Toolkit (GATK). MuTect was applied to the paired plasma DNA BAM files to identify somatic single-nucleotide variants (24). Small insertions and deletions were detected using Scalpel (http://scalpel.sourceforge.net). Purity-adjusted gene-level and segment-level copy number variations (CNVs) were calculated using CNV Kit (25). The tumor mutation burden (TMB) was defined as the number of non-synonymous mutations per sample that had not been previously described as germline alterations. Targetable genes were annotated and confirmed using the ClinVar database; only gene alterations with current clinical Food and Drug Administration (FDA)-approved potential treatment options or late-phase trials for any cancer were identified.

Statistical analysis

Categorical variables were analyzed by Pearson's Chi-squared tests and Fisher's exact test when necessary. Continuous variables were processed by *t*-tests or analysis of variation (ANOVA). Odds ratios were calculated using univariate or multivariable analyses, with 95% confidence interval (95% CI) depicting the different gene alteration frequencies of patients. False discovery rate (FDR) correction used the Benjamini-Hochberg method to adjust P values, thereby controlling the false positive rate while preserving the statistical power of our tests. Survival plots were performed in the Kaplan-Meier method, while differences within groups were assessed by log-rank tests. R software (Version 3.5.3; R Foundation for Statistical Computing, Vienna, Austria), with "ComplexHeatmap", "ggstatsplot", "survival", "survminer" and "tableone" packages was used for analysis. Statistical significance was defined as a two-sided P value less than 0.05.

Results

Cohort overview and genomic profiling

A total of 257 patients meeting the inclusion criteria were

included in the genomic analysis, including 99 GEPNEC, 57 lung NEC, and 101 digestive adenocarcinomas. The flowchart of study and basic information are presented in *Supplementary Figure S1*. First, we investigated the GEPNEC cohort (n=99). The median age of the cohort was 59.6 years old. Small-cell NEC (SCNEC) accounted for 55 (55.6%) patients. Mixed neuroendocrine-non-neuroendocrine neoplasia (MiNEN) accounted for 17.2% of the patients. The median percentage of positive Ki67 was 75%. Stage IV patients accounted for 81.8%, and an elevated NSE level was observed in 65.7% of the patients. The main difference between GEPNEC and LNEC was the relatively balanced proportion of pathology (P<0.001). The origins can be divided into esophagus NEC (ENEC) (n=7), gastric NEC (GNEC) (n=32), duodenal NEC (DNEC) (n=9), colorectal NEC (CRNEC) (n=18), bile tract NEC (BTNEC) (n=8), pancreatic NEC (PNEC) (n=12), and unknown primary NEC (UPNEC) (n=13). The detailed clinicopathological characteristics of the cohort comparisons are summarized in *Table 1*, *Supplementary Table S1*. Pathological distinctions are seen in *Supplementary Figure S2*.

A total of 1,337 alterations were identified (8.57 variants per sample on average, ranging from 1 to 79 variants), including 955 single nucleotide variants (SNVs), 137 insertions and deletions, 183 CNVs, and 62 structure variations (SVs). The top-ranking genes in the whole cohort were *TP53*, *RB1*, *LRP1B*, *KRAS*, *APC*, *TERT*, *CTNNB1*, and *NOTCH1*, with prevalence of 73.7 (115/156), 42.3 (66/156), 20.5 (32/156), 15.4 (24/156), 12.2 (19/156) and 12.2 (19/156), 11.5 (18/156), and 11.5 (18/156), respectively (*Figure 1*).

Genomic comparison of different primary sites of NEC

Considering the distinct pathogenetic and prognosis differences, the genetic profile of GEPNEC was comprehensively interrogated, specifically compared with LNEC (*Table 2*). The comparison showed that GEPNEC had *TP53* (82.8%), *RB1* (38.4%), and *KRAS* mutations (21.2%). The most frequently mutated genes in LNEC were *TP53* (56.1%), *RB1* (49.1%), and *LRP1B* (22.8%). The *TP53*, *KRAS*, *TERT*, *IL7R* and *CTNNB1* had higher alteration frequency in GEPNEC samples than in LNEC samples, while LNEC had typically higher *PIK3CA* and *NKX2-1* amplifications (*Figure 2*). The *TP53* and *RB1* co-mutations had high co-occurrence patterns in LNEC than that in GEPNEC. However, *TERT* and *IL7R* co-amplifications were only seen in GEPNEC (both P<0.01)

Table 1 Overall clinical information of GEPNEC and comparative LNEC cohort

Characteristics	n (%)		P
	GEPNEC (N=99)	LNEC (N=57)	
Age (year) ($\bar{x}\pm s$)	59.6±12.2	61.9±9.8	0.054
Gender			
Female	36 (36.4)	19 (33.3)	0.658
Male	63 (63.6)	38 (66.7)	
Primary sites			–
Bile duct	8 (8.1)	–	
Colorectum	18 (18.2)	–	
Duodenum	9 (9.1)	–	
Esophagus	7 (7.1)	–	
Stomach	32 (32.3)	–	
Pancreas	12 (12.1)	–	
Unknown primary	13 (13.1)	–	
Lung	–	57 (100)	–
Pathology[#]			
Undistinguishable	1 (1.0)	1 (1.8)	<0.001
LCNEC	43 (43.4)	18 (31.6)	
SCNEC	55 (55.6)	38 (66.7)	
Ki67 (%) [Median (IQR)]	75 (60, 80)*	80 (70, 90)	0.072
Mixed component			
MiNEN	17 (17.2)	3 (5.3)	0.032
Pure NEC	82 (82.8)	54 (94.7)	
Stage			
Stage I–III	18 (18.2)	13 (22.8)	0.234
Stage IV	81 (81.8)	44 (77.2)	
Metastases			
Liver	58 (58.6)	17 (29.8)	0.061
Lung	12 (12.1)	–	0.343
Bone	9 (9.1)	9 (15.8)	0.725
NSE elevation			
No	34 (34.3)	17 (29.8)	0.562
Yes	65 (65.7)	40 (70.2)	
Smoking			
No	79 (79.8)	14 (24.6)	<0.001
Yes	20 (20.2)	43 (75.4)	

GEPNEC, gastroenteropancreatic neuroendocrine carcinoma; LNEC, lung NEC; SCNEC, small-cell NEC; LCNEC, large-cell NEC; MiNEN, mixed neuroendocrine-non-neuroendocrine neoplasia; NSE, neuron-specific enolase. [#], one NEC patient had difficulty in identifying the small or large cell subtypes; *, we calculated the Ki67 index of NEC component in MiNEN. In most cases (n=12), the Ki67 index of adenocarcinoma component were concordant with that of NEC component. Only 2 MiNEN cases had different Ki67 (median Ki67 for adenocarcinoma was 20% and for NEC was 80%).

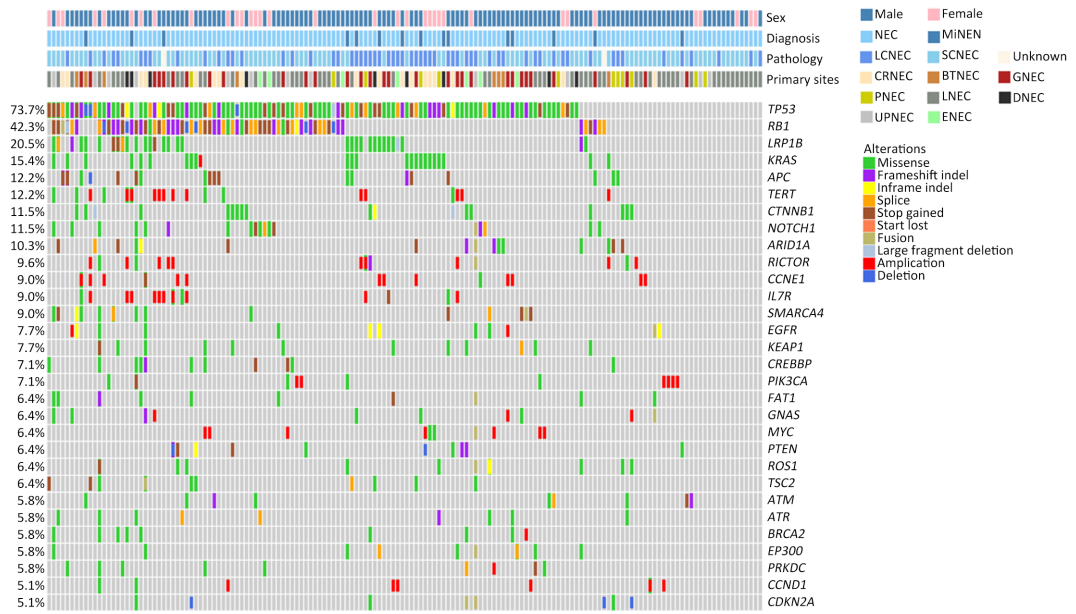

Figure 1 Overview of genomic landscape of whole NEC cohort of GEPNEC and LNEC. NEC, neuroendocrine carcinoma; GEP, gastroenteropancreatic; MiNEN, mixed neuroendocrine-non-neuroendocrine neoplasm; LCNEC, large-cell NEC; SCNEC, small-cell NEC; Unknown, unknown pathological types; CRNEC, colorectal NEC; BTNEC, bile tract NEC; GNEC, gastric NEC; PNEC, pancreas NEC; LNEC, lung NEC; DNEC, duodenum NEC; UPNEC, unknown primary NEC; ENEC, esophagus NEC.

Table 2 Comparisons of significant gene alteration frequency in GEPNEC and LNEC patients

Gene	% (n/N)		OR (95% CI)	P	FDR
	GEPNEC	LNEC			
TP53 (mutation)	82.8 (82/99)	56.1 (32/57)	0.27 (0.12–0.59)	0.001	0.005
NKX2-1 (amplification)	0 (0/99)	10.5 (6/57)	Inf (2.17–Inf)	0.002	0.005
PIK3CA (amplification)	0 (0/99)	10.5 (6/57)	Inf (2.17–Inf)	0.002	0.005
KRAS (mutation)	21.2 (21/99)	3.5 (2/57)	0.14 (0.01–0.60)	0.002	0.005
TERT (amplification)	13.1 (13/99)	0 (0/57)	0 (0–0.52)	0.002	0.006
IL7R (amplification)	10.1 (10/99)	0 (0/57)	0 (0–0.73)	0.014	0.023
CTNNB1 (mutation)	16.2 (16/99)	3.5 (2/57)	0.19 (0.02–0.86)	0.019	0.027
GRIN2A (mutation)	1.0 (1/99)	8.8 (5/57)	9.29 (1.00–449.03)	0.025	0.028
PTPRD (mutation)	1.0 (1/99)	8.8 (5/57)	9.29 (1.00–449.03)	0.025	0.028
RB1 (mutation)	38.4 (38/99)	49.1 (28/57)	1.48 (0.95–8.46)	0.042	0.050

GEPNEC, gastroenteropancreatic neuroendocrine carcinoma; LNEC, lung NEC; OR, odds ratio; 95% CI, 95% confidence interval; FDR, false discovery rate; Inf and −Inf, infinitive and −infinitive.

(*Supplementary Figure S3*). The commonly-seen genes ever reported are summarized in *Supplementary Table S2*. We conducted a Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis and concentrated on the major aberrant pathways: PI3K/Akt, Ras/Raf, cell cycle, Wnt and Notch. We then compared their roles between GEPNEC and LNEC. Pathway alterations of Wnt and MAPK signaling pathways were significantly enriched in GEPNEC (*Supplementary Figure S4*). Furthermore, 42.4% (42/99) of GEPNEC had higher amplification of *RB1*, *APC*, *PTPRD*, *CDKN2A*, *PTEN*, and *TP53*. In contrast, 52.6% (30/57) of LNEC had CNVs. The LNEC suggested higher CNV differences of resulted mainly in increased *PIK3CA* and *NKX2-1* amplification (*Supplementary Figures S5*,*S6*). When the genetic profiles of specific primary sites were compared with LNEC, we found that although *TP53* mutation was prevalently observed in each subgroup of GEPNEC, each site had its features. In GNEC, *TERT* amplification was the

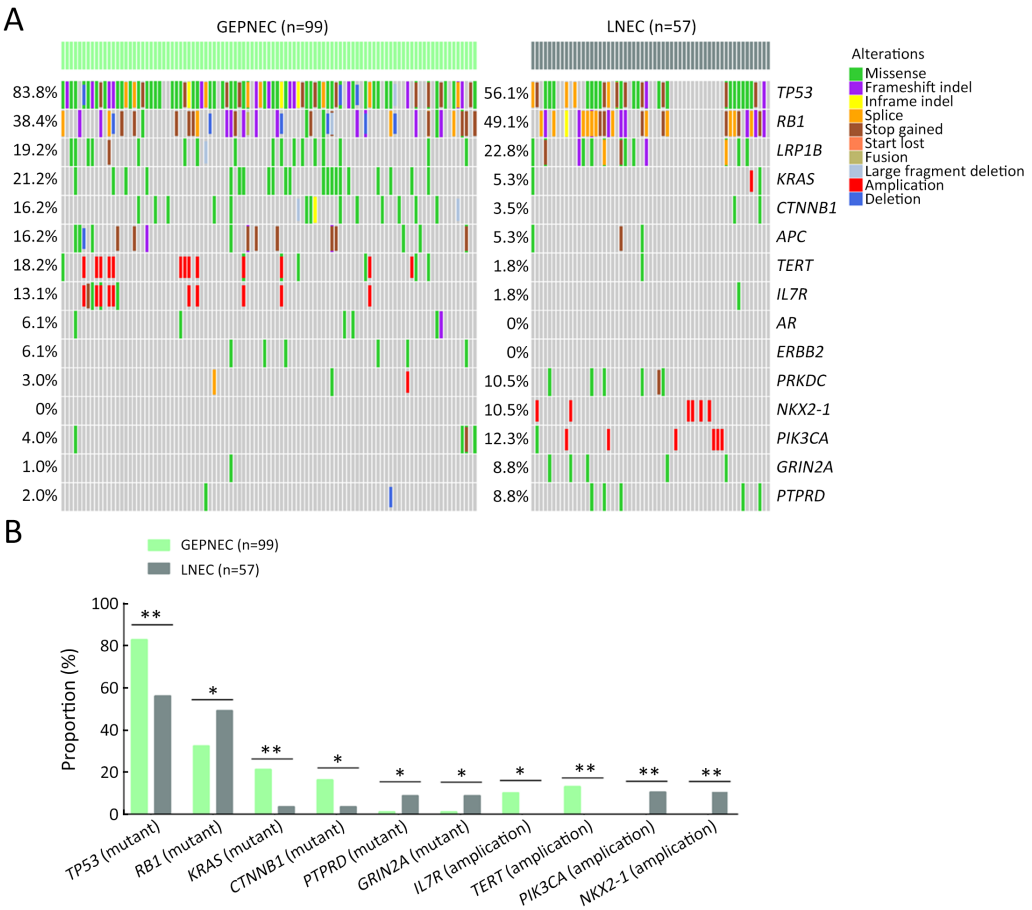

Figure 2 Comparisons of significant gene alteration frequency of GEPNEC and LNEC patients. (A) Oncoprints showing the top most frequent and significant gene alterations between GEPNEC and LNEC; (B) Bar plots of pathway alteration proportions of GEPNEC and LNEC. GEPNEC, gastroenteropancreatic neuroendocrine carcinoma; LNEC, lung NEC. P values were determined using Fisher's exact test. *, P<0.05; **, P<0.01.

most significantly altered gene (28.1% *vs.* 0, P<0.001), and *RB1* mutation was relatively lower than in LNEC (28.1% *vs.* 49.1%, P=0.033). CRNEC had markedly higher mutations of *TP53* (94.4% *vs.* 56.1%, P=0.004), *APC* (33.3% *vs.* 5.3%, P=0.005) and *KRAS* (44.4% *vs.* 3.5%, P=0.048) compared with LNEC. Of other categories, DNEC had higher *TERT* amplification, *KRAS* mutation, and *IL7R* amplification (P<0.05). BTNEC had higher *CTNNB1* (37.5% *vs.* 3.5%, P=0.011) and *ARID1A* (50.0% *vs.* 5.3%, P=0.003) mutations than LNEC. ENEC had higher *SETD2* mutation rate than LNEC (28.6% *vs.* 0, P=0.010) (*Supplementary Table S3*). Major genomic differences between sub-category of GEPNEC and LNEC can be seen in *Supplementary Figure S7*.

Genomic comparison of different morphology

Molecular differences between SCNEC (n=93), including 55 GEPNEC and 38 LNEC, and LCNEC (n=61), including 43 GEPNEC and 18 LNEC, had less heterogeneity than the differences in the primary sites. *KEAP1*, *FANCM*, *TP53*, *CDH1* and *KIT* mutations were more enriched in LCNEC compared with SCNEC (*Supplementary Figure S8*). Furthermore, when compared to GEPSCNEC, GEPLCNEC only suggested higher mutation frequency of *CDH1* and *FANCM* (*Supplementary Figure S9*, *Supplementary Table S4*). The CNV frequency of LCNEC was comparable to SCNEC (*Supplementary Figure S5*). We also compared the differences between MiNEN (n=17) and pure NEC (n=82) to see if mixed component influenced the result. MiNEN appeared to have no significantly different genes than pure NEC in our cohort (*Supplementary Table S5*).

Genomic comparison of GEPNEC to local adenocarcinoma

The differential gene alteration analysis of GEPNEC and

local adenocarcinoma is shown in *Figure 3*. When compared with digestive adenocarcinoma, GEPNEC had a higher frequency of *RB1* mutation [31.3% (31/99) *vs*. 7.9% (8/101), P<0.001] and lower frequencies of *APC* mutation [16.2% (16/99) *vs*. 41.6% (42/101), P=0.011] and *KRAS* mutations [17.2% (17/99) *vs.* 44.6% (45/101), P<0.001] (*Table 3*). When comparing GEPNEC and digestive adenocarcinoma by primary sites, we found that GNEC had higher *TP53* and *RB1* mutations than gastric adenocarcinoma (P<0.05). In contrast, *ARID1A*, *PI3KCA*, and *LRP1B* had significantly lower alteration frequencies (P<0.05). CRNEC had a remarkably higher *RB1* mutation rate [38.9% (7/18) *vs*. 10.7% (6/56), P<0.001] than colorectal adenocarcinoma. In PNEC, *KRAS* mutation frequency [16.7% (2/12) *vs.* 75.0% (9/12), P=0.034] was significantly lower than pancreatic ductal adenocarcinoma (*Supplementary Table S6*).

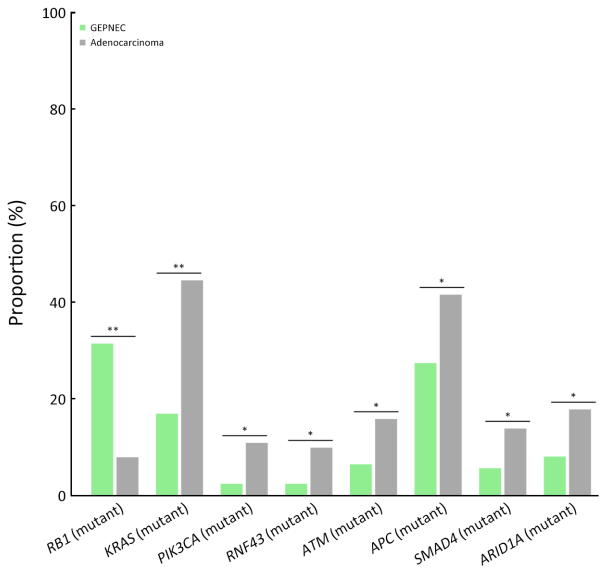

Figure 3 Bar plots of comparisons of significant gene alteration frequency of GEPNEC and local adenocarcinoma patients. GEPNEC, gastroenteropancreatic neuroendocrine carcinoma. *, P<0.05; **, P<0.01.

Therapeutic investigation of GEPNEC

We explored the relations between responses of first-line regimens and *TP53*/*RB1* co-alteration patterns (deemed as SCLC-like GEPNEC) and other patterns (deemed as non-SCLC-like GEPNEC) in advanced GEPNEC from our cohort. We found that SCLC-like patients were prone to benefit from etoposide and platinum (EP) chemotherapy, but SCLC-like patients had less benefits from adenocarcinoma chemotherapy (*Table 4*).

Targetable genes with FDA-approved drugs were specifically identified in 12 GEPNEC patients. Two patients had $BRAF^{V600E}$ mutations, and one had a $KRAS^{G12C}$ mutation. *CDK4* and *CDK6* mutations emerged as putative targets in one patient. *ALK* and *MET* amplifications were seen in two patients. Mutation frequency of targetable $PIK3CA^{E545K}$ mutation (1.0%, 1/99) of GEPNEC was significantly lower than that of LNEC. Approximately 3.0% (3/99) had germline alterations in the DNA damage repair (DDR) pathway, including *BRCA2*, *ATM*, and *ATR* mutations (*Table 5*). Microsatellite instability (MSI) was also observed in 4.4% of GEPNEC, including two CRNEC (11.1%) patients, one GNEC, and one PNEC patient. The median TMB of GEPNEC was 5.3 mutations/Mb. GNEC had a median TMB of 6.5 mutations/Mb (Q1–Q3: 4.2–7.6), CRNEC had a median TMB of 6.3 mutations/Mb (Q1–Q3: 1.7–6.7), PNEC had a median TMB of 1.7 mutations/Mb (Q1–Q3: 0.9–2.1), and

Table 3 Comparisons of significant gene alteration frequency of GEPNEC and local adenocarcinoma patients

Gene	% (n/N)		OR (95% CI)	P	FDR
	GEPNEC *vs.* Adenocarcinoma				
RB1 (mutation)	31.3 (31/99)	7.9 (8/101)	6.12 (2.65–16.00)	<0.001	<0.001
KRAS (mutation)	17.2 (17/99)	44.6 (45/101)	0.26 (0.13–0.49)	<0.001	<0.001
PIK3CA (mutation)	2.0 (2/99)	10.9 (11/101)	0.20 (0.04–0.80)	0.018	0.036
RNF43 (mutation)	2.0 (2/99)	9.9 (10/101)	0.23 (0.04–0.92)	0.033	0.053
ATM (mutation)	6.1 (6/99)	15.8 (16/101)	0.37 (0.13–0.96)	0.040	0.053
APC (mutation)	16.2 (16/99)	41.6 (42/101)	0.53 (0.29–0.96)	0.011	0.029
SMAD4 (mutation)	7.1 (7/99)	13.9 (14/101)	0.37 (0.12–1.00)	0.166	0.166
ARID1A (mutation)	8.1 (8/99)	17.8 (18/101)	0.41 (0.16–0.98)	0.057	0.065

GEPNEC, gastroenteropancreatic neuroendocrine carcinoma; OR, odds ratio; 95% CI, 95% confidence interval; FDR, false discovery rate.

Table 4 Association of gene-base subtypes of GEPNEC* and first-line regimens

GEPNEC types	Regimens	% (n/N)	
		ORR	DCR
Genomic-based subtype			
SCLC-like	EP	50.0 (11/22)	81.8 (18/22)
	Other	0 (0/5)	40.0 (2/5)
Non-SCLC-like	EP	18.9 (7/37)	73.0 (27/37)
	Other	33.3 (4/12)	75.0 (9/12)
Pathological-based subtype			
SCNEC	EP	30.8 (12/39)	82.0 (32/39)
	Other	33.3 (2/6)	50.0 (3/6)
LCNEC	EP	25.0 (3/12)	58.3 (7/12)
	Other	21.1 (4/19)	63.2 (12/19)

GEPNEC, gastroenteropancreatic neuroendocrine carcinoma; ORR, overall response rate; DCR, disease control rate; SCLC, small-cell lung cancer; SCNEC, small-cell NEC; LCNEC, large-cell NEC; EP, etoposide and platinum; *, among stage IV, 76 patients had recorded first-line therapy. SCLC-like type, GEPNEC patients with *TP53* and *RB1* co-mutation (n=27); Non-SCLC-like, GEPNEC patients except for SCLC-like type (n=49); Other regimens mainly included irinotecan-based or oxaplatin-based therapy, which were commonly applied in gastrointestinal adenocarcinoma.

UPNEC had a median TMB of 7.4 mutations/Mb (Q1–Q3: 4.3–15.6) (*Supplementary Figure S7*). When accounting for MSI-H and TMB-high into targetable patients, the total number came to 22.2% (22/99). We found that these patients receiving corresponding second-line target therapies had significantly longer PFS [12.5 months *vs.* 3.0 months, HR=0.40 (0.21–0.75), P=0.006] and OS [46.9 months *vs.* 13.4 months, HR=0.30 (0.11–0.73), P=0.020] than non-targetable patients (*Figure 4*).

Discussion

Currently, management for GEPNEC follows the guidelines of SCLC, regardless of primary site and pathology. However, some patients still had rapid disease progression (26,27). Current genomic studies mainly focus on NENs (7), whereas genetic studies on GEPNEC are relatively scarce (4,28). This study explored the genomic features of GEPNEC compared with different primary sites and pathology.

In this study, GEPNEC differed from SCLC and had fewer driver gene patterns of SCLC, like *TP53*/*RB1* co-mutations. Besides the prevalence of *TP53* mutations (32.9%–83.8%) (7,29-31), our results were consistent with what the various anatomic sites revealed from large-sample studies (12,32). The low *RB1* mutation rate might be associated with some race differences between the incidence of *RB1* mutations in Asians and that in Caucasians (33). Besides, some previous results primarily relied on IHC data, where molecular events (like methylation and deletions) affecting *RB1* protein expression may be attributed to discordance. Gene variations between GEPNEC and LNEC were *CTNNB1*, *TERT*, and *IL7R*. Notably, this study revealed that amplifications of *TERT* and *IL7R* were unique alterations in GNEC, yet the mechanisms of their co-amplification remain unknown (7).

Table 5 Potential treatment targets through ClinVar in GEPNEC

Target genes	No.	Drugs
BRAF^{V600E} (mutation)	2	Vemurafenib, Dabrafenib, Encorafenib
MET (amplification)/*ALK* (fusion)	2	Crizotinib, Lorlatinib, Ceritinib, Alectinib, Brigatinib
KRAS^{G12C} (mutation)	1	Sotorasib, Adagrasib, JNJ-74699157, GDC-6036, LY3499446, JDQ443, D-1553
RET (fusion)	2	Selpercatinib, Pralsetinib, Cabozantinib, Vandetanib, TPX-0046, RXDX-105, BOS172738
PIK3CA^{E545K} (mutation)	1	Everolimus, Temsirolimus, Alpelisib, Copanlisib, Taselisib, CYH33, TQ-B3525, BKM120, Inavolisib, SF1126, GDC-0084, Copanlisib hydrochloride
CDK4/*CDK6* (amplification)	1	Palbociclib, Ribociclib, Abemaciclib, SHR6390, PD 0332991, Trilaciclib, G1T38, LEE011
CDH1 (mutation)	1	Crizotinib, Savolitinib, Capmatinib, Tepotinib, ARQ 197
BRCA12, *ATM* and *ATR* (germline mutation)	3	Olaparib, Rucaparib, Niraparib, Talazoparib
MSI-H	3	Pembrolizumab, Nivolumab, Ipilimumab, Tislelizumab, Sintilimab, Toriplimab, Camrelizumab, Envafolomab
TMB-H	16	

GEPNEC, gastroenteropancreatic neuroendocrine carcinoma; Genes associated with target therapy were listed binding to the applications of potential drugs. Drugs were approved by Food and Drug Administration (FDA) or used in phase III clinical trial.

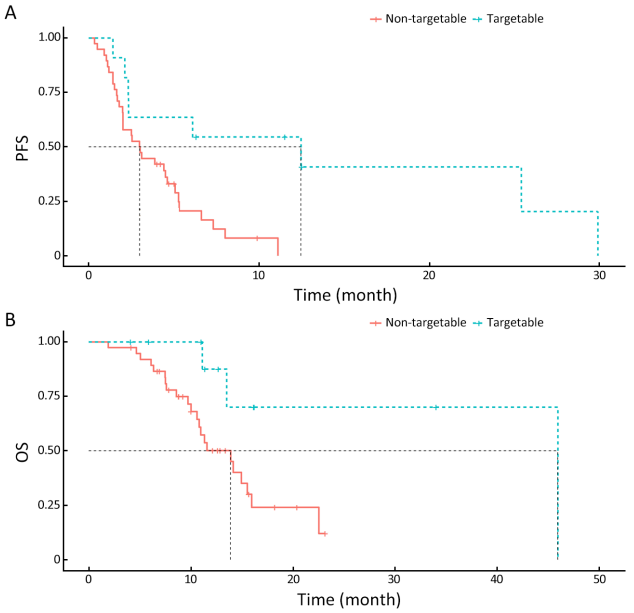

Figure 4 Survival plots of targetable patients with target therapy and non-targetable patients with other therapies. (A) PFS of patients with/without target therapy (P=0.006); (B) OS of patients with/without target therapy (P=0.020). PFS, progression-free survival; OS, overall survival.

TERT promoter mutations were found in PNET/LNET as potential clinical implications. The PI3K/Akt, Ras/Raf, Wnt, and Notch signaling pathways were affected by mutations in GEPNEC and LNEC and appeared to play a paramount role in GEPNEC. These mutations were closely related to carcinogenesis and might play important roles in the development of cancer.

Specific genes of GEPNEC compared with LNEC can be analyzed by primary sites. A small-sample study focusing on GNEC confirmed that the gene alterations were similar to adenocarcinoma but had different spectrums of *HER2* (12,14,32). The study found that 8.3% of GNEC patients presented with *HER2* over-expression, and 16.7% presented with a loss of E-cadherin. Generally, GNEC may share similar molecular spectrums with stomach adenocarcinoma, as proposed by Ishida *et al*. (34). Koh *et al*. uncovered common genetic spectrums between two components of gastric MiNEN. CRNEC had *KRAS* (8.3%–60%), *APC* (4.2%–80%), and *BRAF* (4.2%–58.6%) mutations as the key differential genes from other GEPNECs (10,15,16,20,35). CpG island methylator phenotype (CIMP) and *BRAF* mutations also emerged as clinical biomarkers in colorectal cancers and are waiting for further validation in CRNEC (13,36,37). PNEC demonstrated different genetic profiles, with a relatively lower *TP53/RB1* mutation frequency. While higher *ROS1*, *CDKN2A*, and *KRAS* mutations (25%) were observed in PNEC when compared with gastrointestinal NEC (GINEC) (16,38). These specific genomic patterns support the closer pathogenetic relationship between PNEC and non-neuroendocrine pancreatic carcinoma than G3 NET (39). PNEC can be divided into "Ductal-type" and "Acinar-type" by WES/WGS, characterized by *KRAS/TP53/RB1* and *CTNNB1/CDKN2A/TP53* mutations, which further clarifies the molecular classification of GEPNEC (6). ENEC exhibits a higher frequency of *NOTCH1* mutation, which may serve as a distinguishing factor. The gene spectrum of DNEC emerged as the overlapping entity that bridged GNEC and CRNEC. Further mechanisms may be associated with the location of transition between the upper and lower gastrointestinal tracts. For BTNEC, we confirmed that *ARID1A* mutations were common, which is associated with immunotherapy in NEN (40).

Our findings suggest that the genetic differences from morphology (small-cell *vs.* large-cell) were relatively small, and stratification may not reflect the features well. The molecular subtypes of SCLC have been well established, and novel classifications of LCNEC may supplement more therapeutic utility. A previous study highlighted that SCLCs were genetically different from LCNEC in alterations and CNV spectra (41). We observed that 30%–40% of previous sequencing was comprised of non-neuroendocrine components, which could confound the findings related to genuine neuroendocrine malignancies (11,15,17,34). Some studies have suggested that NEC variants may originate from autochthonous non-NECs of these sites (12,18,39). MiNEN had no marked gene differences compared with pure NEC, perhaps due to the relatively small number. It is important to distinguish between pure NEC and mixed tumors in future analysis.

Therapeutics may be guided in the light of the genome, regardless of comorbidity and pathology. The alteration frequencies of GEPNEC were not comparable to the average levels of pan-cancer (20.9% *vs.* 57.0%). Although some studies have reported that 94% of NECs had putative responsive biomarkers to targetable mutations or ICI, but the criteria were not as strict for the targetable genes (8). Current targetable drugs of GEPNEC markedly differed from those of LNEC. Further evidence on GEPNEN uncovered that 49% of advanced NENs harbored actionable genomic alterations. Vijayvergia *et al*. revealed a spectrum of targetable genes ranked by *PIK3CA/PTEN* (22%) and *BRAF* (13%) mutations (7), with *BRAF*

mutations found in 20% of GEPNECs (5). Additionally, *PI3KCA* mutations (4.9%−12.5%) have a potential role in targeting the *PI3K/AKT* signaling pathway, while *KRAS* and *BRAF* mutations influenced the prognosis of GEPNEC (42). The BRAF inhibitors (widely used for adenocarcinoma) may also be used for CRNEC (35,43). We observed that 2/18 of CRNECs had a $BRAF^{V600E}$ mutation in our study, which was relatively low compared to the range of incidence in CRNEC (4.1%−88.2%) (9,11,15,30,37). These mutations could be confirmed in prominent actionable mutations in the Wnt pathway, where the proportions of *APC* (14.3%−17.5%) and *CTNNB1* (16.4%) mutations were appreciable (7). *CDK4/CDK6* amplification was seen in five patients. Olaparib has shown promising efficacy with an overall response rate (ORR) of 41.7% in SCLC with similar alterations (44).

GEPNECs from different origins showed relatively low TMB levels, consistent with a previous report on extrapulmonary NECs (20,45). TMB-H was unrelated to longer OS in GEPNEC, but we observed a correlation between TMB-H and longer PFS (over 16 months) upon using second-line anti-PD-1 therapy in our cohort. Lu *et al*. confirmed that TMB-H demonstrated good efficacy in extrapulmonary NEN (40). MSI and PD-L1 statuses were also associated with a better prognosis and response in GEPNEC (46-48). A previous study reported the variable frequency range of MSI distribution in GEPNEC of 0−69.7%, which was also prevalent in GNEC and CRNEC (13,49,50). However, it remains undetermined whether these identified potential druggable genomic alterations could be translated into clinical practice (6). Here, we demonstrated that the driver genes of GEPNEC had putative roles in improving the clinical outcomes, and the relevance of these genes for target/immunotherapy warrants further study. Since targeted and immune therapies offer additional and effective treatment options for SCLC, basket trials can be employed to identify more individualized therapeutic options in GEPNEC (51).

We also explored the relations between the response of first-line EP regimens and "SCLC-like" GEPNEC and revealed the positive relevance. This intriguing result may be attributed to the genetic nature of the *TP53/RB1* co-mutation pattern reflecting the sensitivity to the regimens, similar to SCLC. Some LCNEC with a *TP53/RB1* co-mutation responded better to EP (41,52). These findings emphasize the potential for targeted therapies based on the genomic profile of GEPNEC, highlighting specific genes and signaling pathways that could be therapeutically exploited.

However impactful these results are, there were some limitations. First, the sample size was relatively small, which may limit the generalizability of these findings. In addition, the ratio of the different pathological subtypes differed compared with the reported world ratios. Stratifications according to the molecular context should be the pursuit of precise medication to contribute to optimal therapeutic options; however, the small sample size may hinder comprehensive analyses in this regard. Moreover, the NGS panel we used only covered 233 genes, limiting the accuracy of the TMB evaluations (53). A broader gene panel could provide a more comprehensive assessment of genomic alterations and TMB levels.

Conclusions

The genetic features of GEPNEC are distinct from those of LNEC and local adenocarcinoma. Several key genetic alterations differentiate GEPNEC from LNEC and local adenocarcinoma, including *RB1*, *KRAS*, *APC*, *TERT*, *ARID1A*, and *CTNNB1*. In comparison with LNEC, GEPNEC shows differences in *RB1* and *KRAS* mutations. GEPNEC and digestive adenocarcinoma had differences in *RB1* and *KRAS* mutation as well. Specifically, the following GEPNEC subgroups had particular alterations: GNEC (*TERT* amplification), CRNEC (*KRAS* mutation), and BTNEC (*ARID1A* mutation). Targetable genes were identified in approximately 22.2% of the GEPNEC patients and demonstrated promising PFS outcomes. We believe in promising future that genes may guide GEPNEC treatment, but further attempts in genetic analysis are needed to elucidate the underlying mechanisms, and large-sample prospective studies are warranted to validate these findings.

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Footnote

Conflicts of Interest: Junli Zhang, Sha Wang and Yang Shao are the employees of Geneseeq Technology, Inc. Yanfang Guan and Wenguang Gu are the employees of Geneplus-Beijing, Inc. The other authors have no conflicts of interest to declare.

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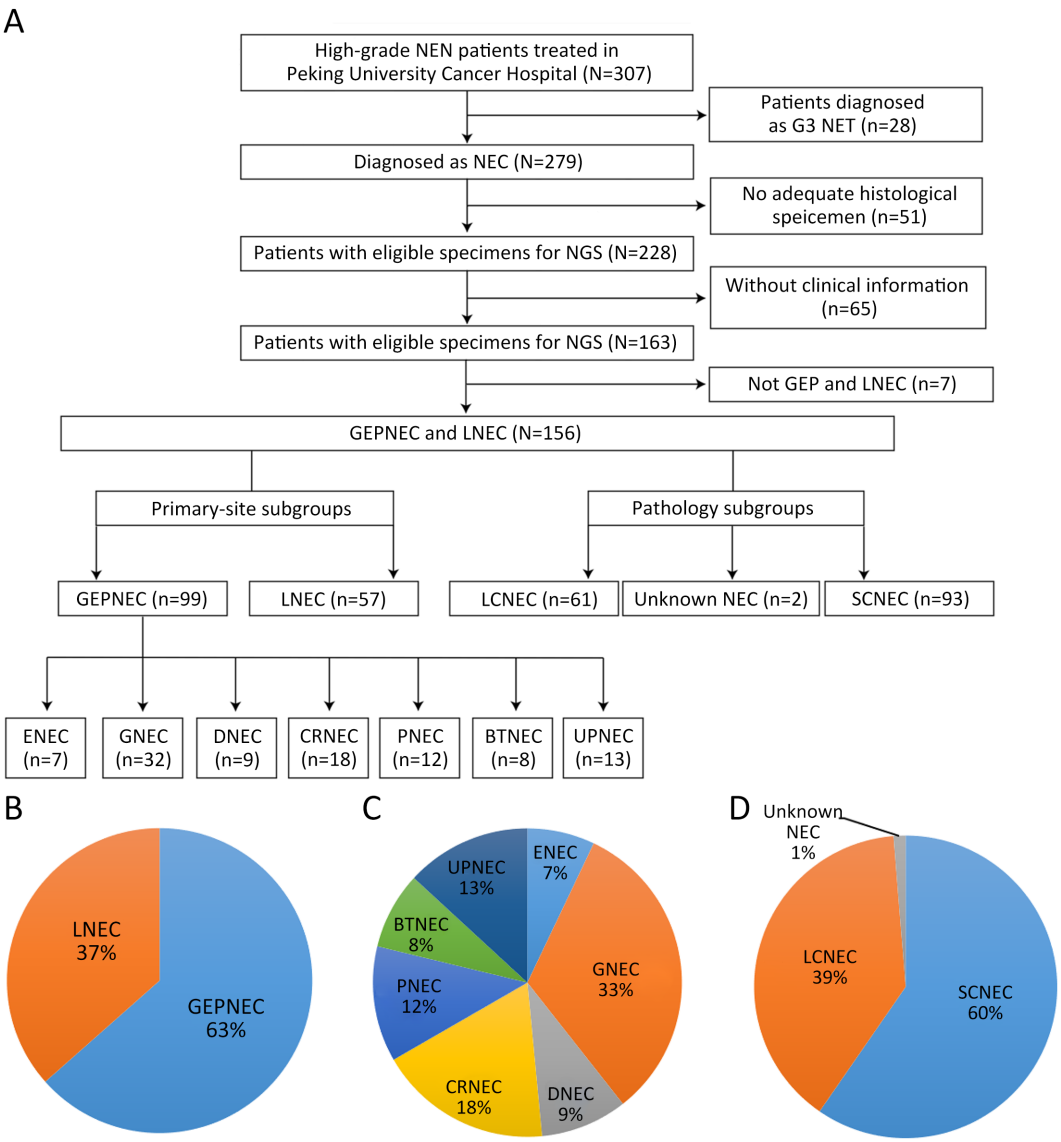

Figure S1 Overview of flowchart in study inclusion process and pie charts of basic subgroups of NEC. (A) Flowchart of whole study; (B) Primary tumor sites; (C) Detailed primary tumor sites; (D) Pathology subgroups. NEN, neuroendocrine neoplasm; NET, neuroendocrine tumor; NEC, neuroendocrine carcinoma; GEP, gastroenteropancreatic; LNEC, lung NEC; LCNEC, large-cell NEC; SCNEC, small-cell NEC; ENEC, esophagus NEC; GNEC, gastric NEC; DNEC, duodenum NEC; CRNEC, colorectal NEC; PNEC, pancreas NEC; BTNEC, bile tract NEC; UPNEC, unknown primary NEC.

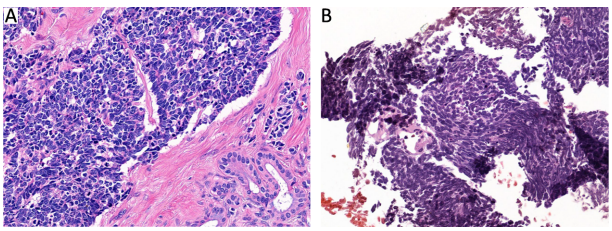

Figure S2 Morphological distinctions between GEPNEC and LNEC. (A) SCNEC of PNEC; (B) SCNEC of lung. GEPNEC, gastroenteropancreatic neuroendocrine carcinoma; LNEC, lung NEC; SCNEC, small-cell NEC; PNEC, pancreatic NEC. Hematoxylin-eosin staining, ×400.

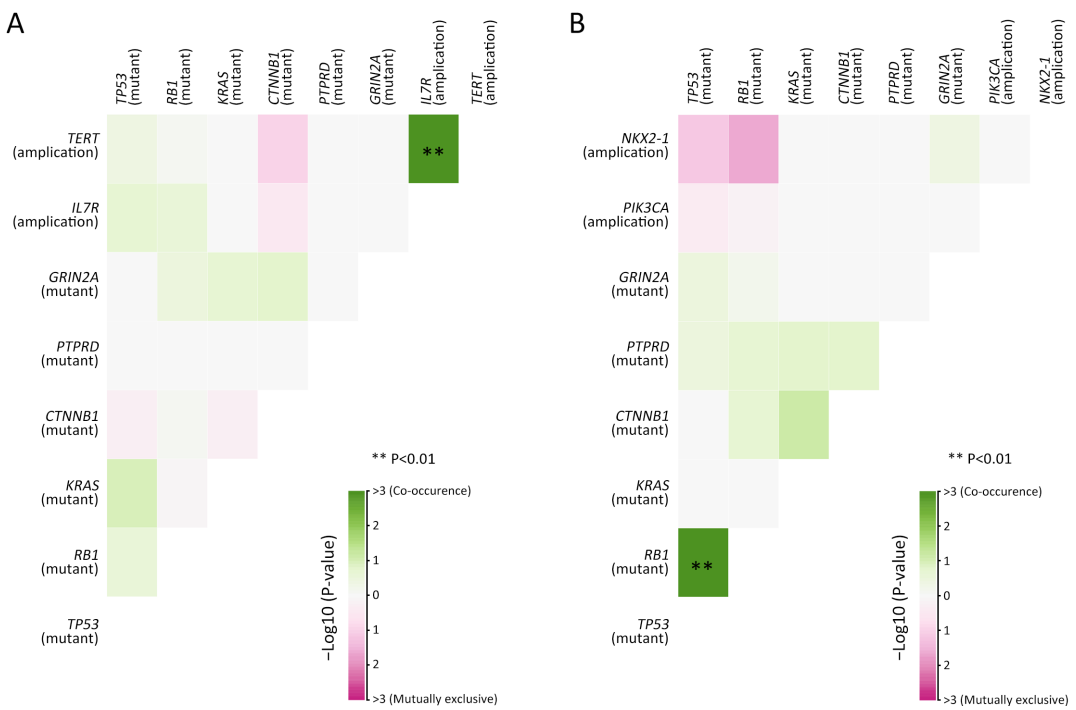

Figure S3 Correlations analysis of co-occurrence genes in GEPNEC and LNEC. (A) Co-occurrence and mutual exclusiveness of gene alteration pattern in GEPNEC patients; (B) Co-occurrence and mutual exclusiveness of gene alteration pattern in LNEC patients. GEPNEC, gastroenteropancreatic neuroendocrine carcinoma; LNEC, lung NEC. Co-occurring mutations are indicated by green squares and mutually exclusive mutations between gene pairs in purple. The color intensity is proportionate the –log10 (P-value). P values were determined using Fisher's exact test.

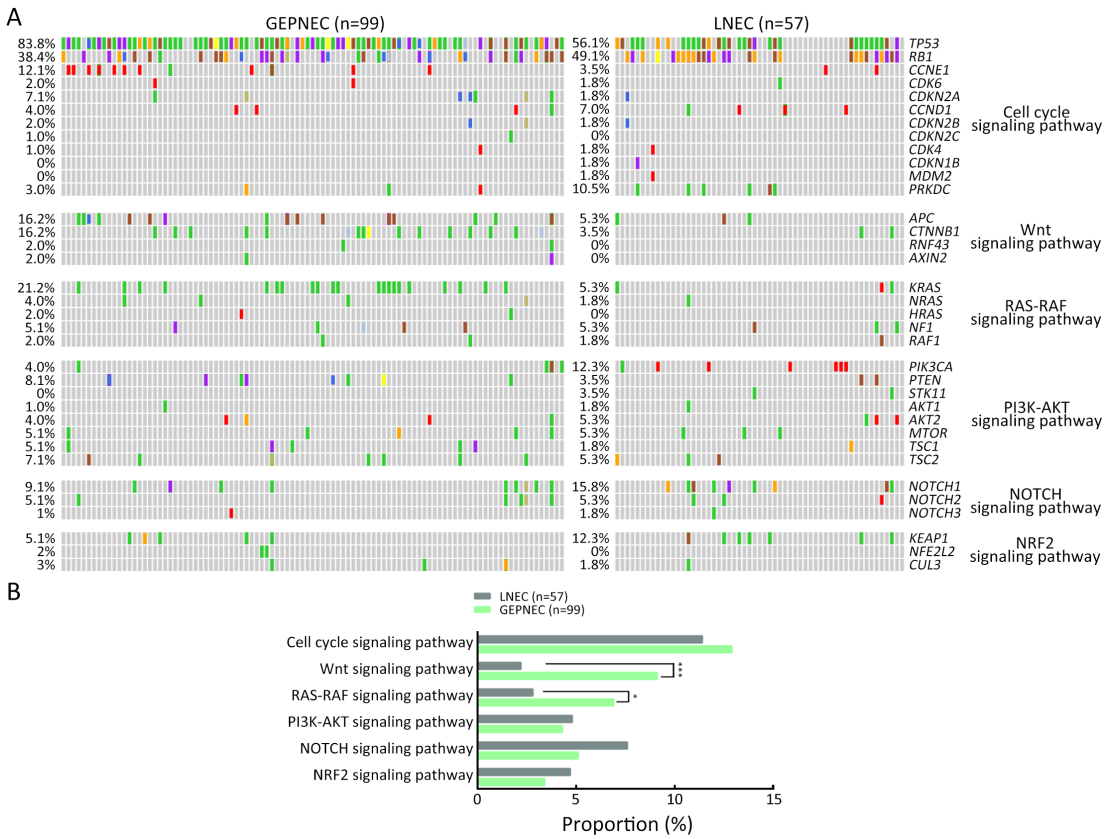

Figure S4 Summary of gene pathway analysis of GEPNEC *vs*. LNEC. (A) Oncoprints of main pathways with alteration frequency among GEPNEC and LNEC patients; (B) Bar plots of pathway alteration proportions of GEPNEC *vs*. LNEC. GEPNEC, gastroenteropancreatic neuroendocrine carcinoma; LNEC, lung NEC. P values were determined using Fisher's exact test. *, P<0.05; ***, P<0.001.

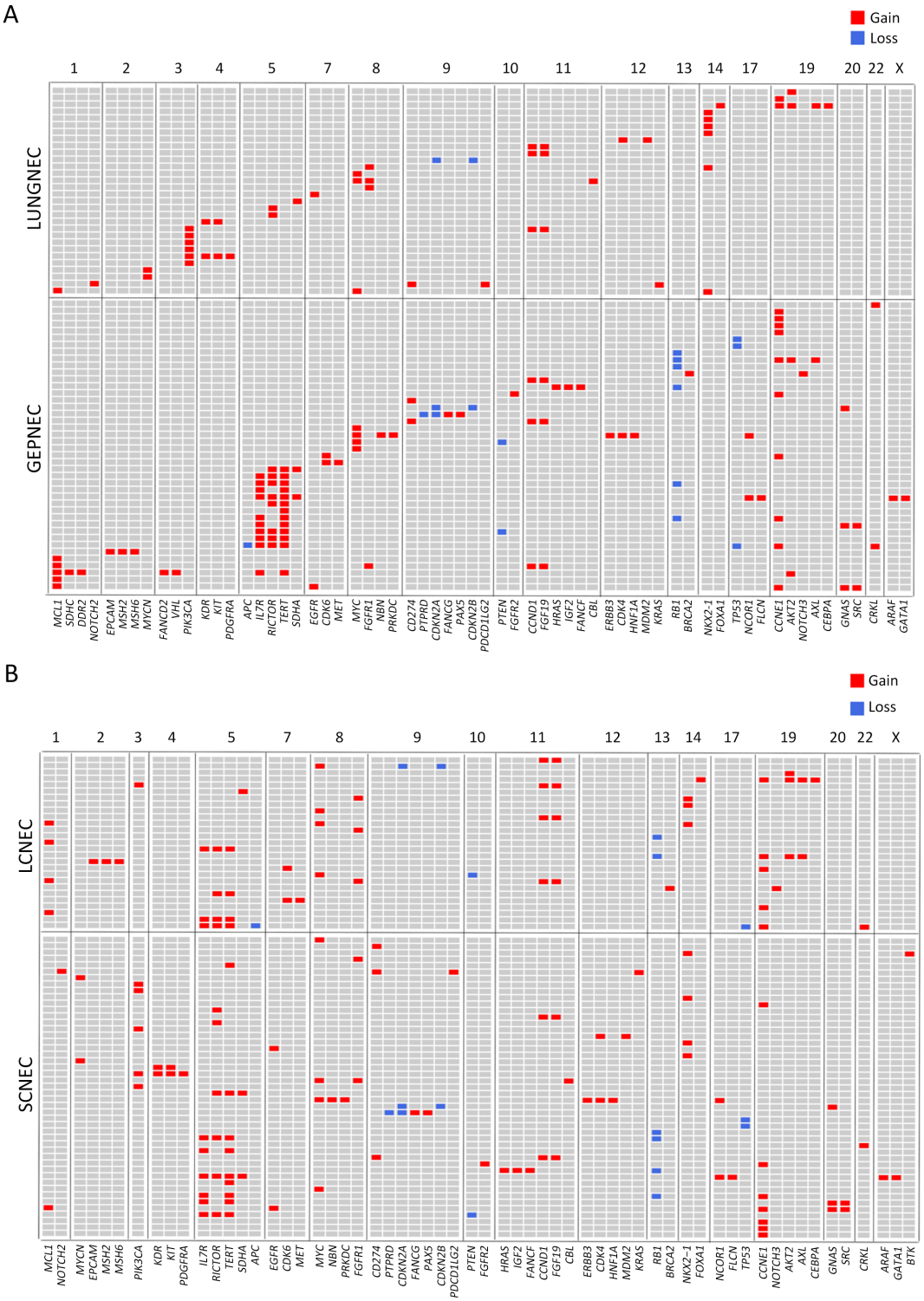

Figure S5 CNV analysis between GEPNEC and LNEC. (A) Graphics representing CNV data by sites of origins. The gain or loss of CNV regions in each clade is labeled with colored boxes. Red, gain; blue, loss; (B) Graphics demonstrating CNV data through pathology. GEPNEC, gastroenteropancreatic neuroendocrine carcinoma; LNEC, lung NEC; CNV, copy number variation.

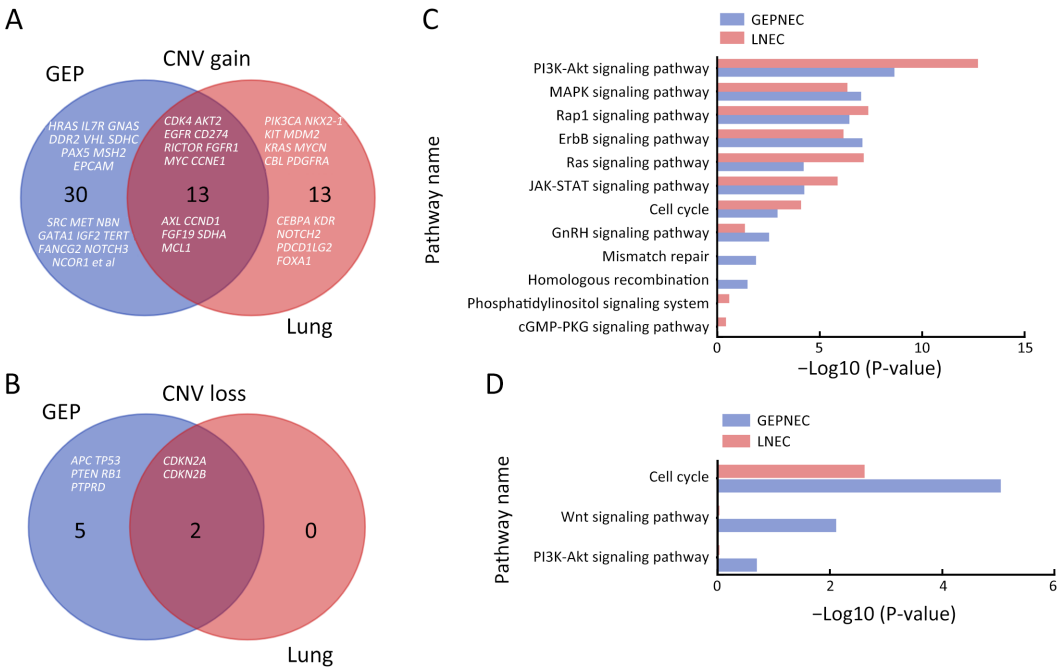

Figure S6 Distinctions of CNV gain/loss between GEPNEC and LNEC. (A,B) Venn plots for CNV gain (A) and CNV loss (B) of genes between GEPNEC and LNEC; (C,D) Bar plots showing quantity analysis of CNV in gene pathway CNV gain (C) and CNV loss (D). CNV, copy number variation; GEPNEC, gastroenteropancreatic neuroendocrine carcinoma; LNEC, lung NEC.

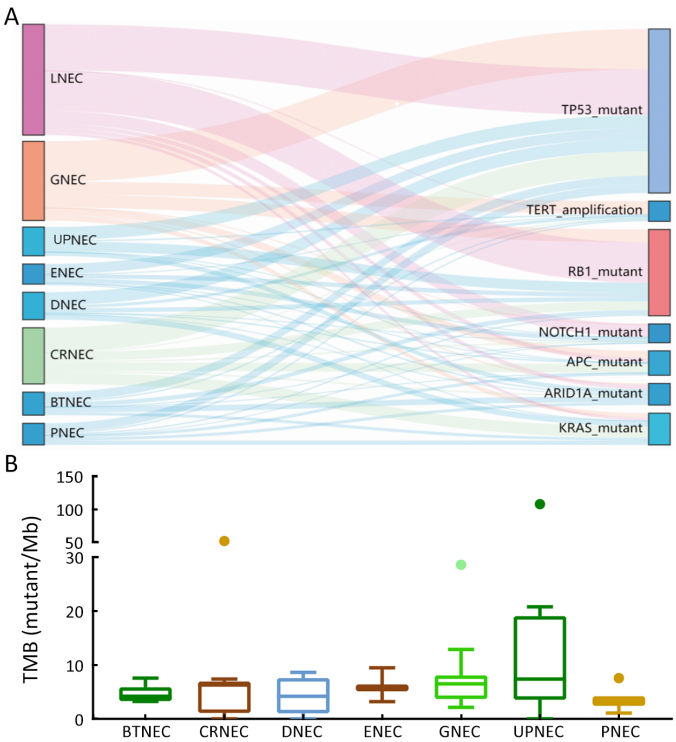

Figure S7 Drivers genes and TMB distributions for GEPNEC of different primary sites. (A) Sankey plots of distinct driver genes of high frequency in GPENEC with different locations; (B) Box plots of TMB of various primary sites of GEPNEC. TMB, tumor mutation burden; GEPNEC, gastroenteropancreatic neuroendocrine carcinoma; LNEC, lung NEC; GNEC, gastric NEC; UPNEC, unknown primary NEC; ENEC, esophagus NEC; DNEC, duodenum NEC; CRNEC, colorectal NEC; BTNEC, bile tract NEC; PNEC, pancreas NEC.

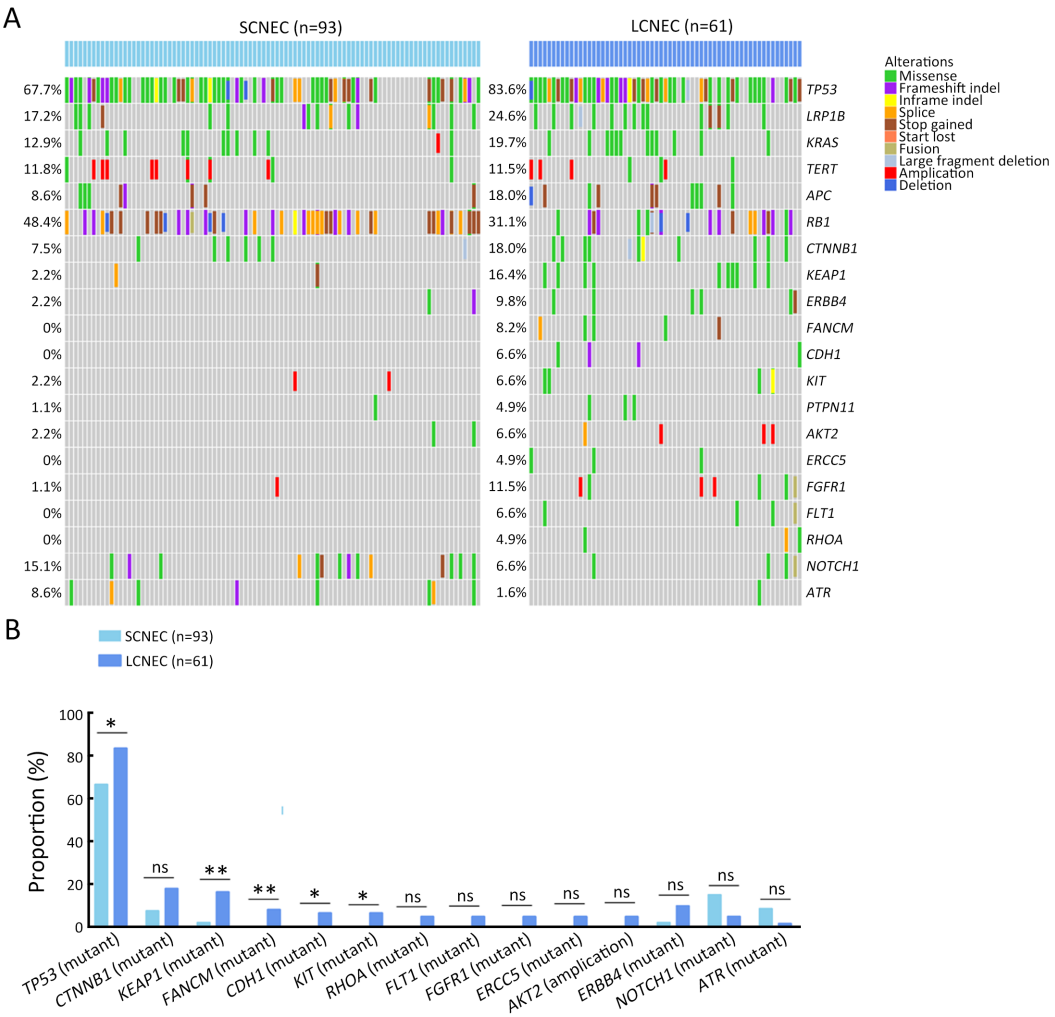

Figure S8 Summary of alteration frequency of SCNEC *vs*. LCNEC. (A) Oncoprints showing the top most frequent and significant gene alterations between SCNEC and LCNEC; (B) Bar plots of pathway alteration proportions of SCNEC and LCNEC. SCNEC, small-cell neuroendocrine carcinoma; LCNEC, large-cell neuroendocrine carcinoma. P values were determined using Fisher's exact test. *, P<0.05; **, P<0.01; ns, no significance.

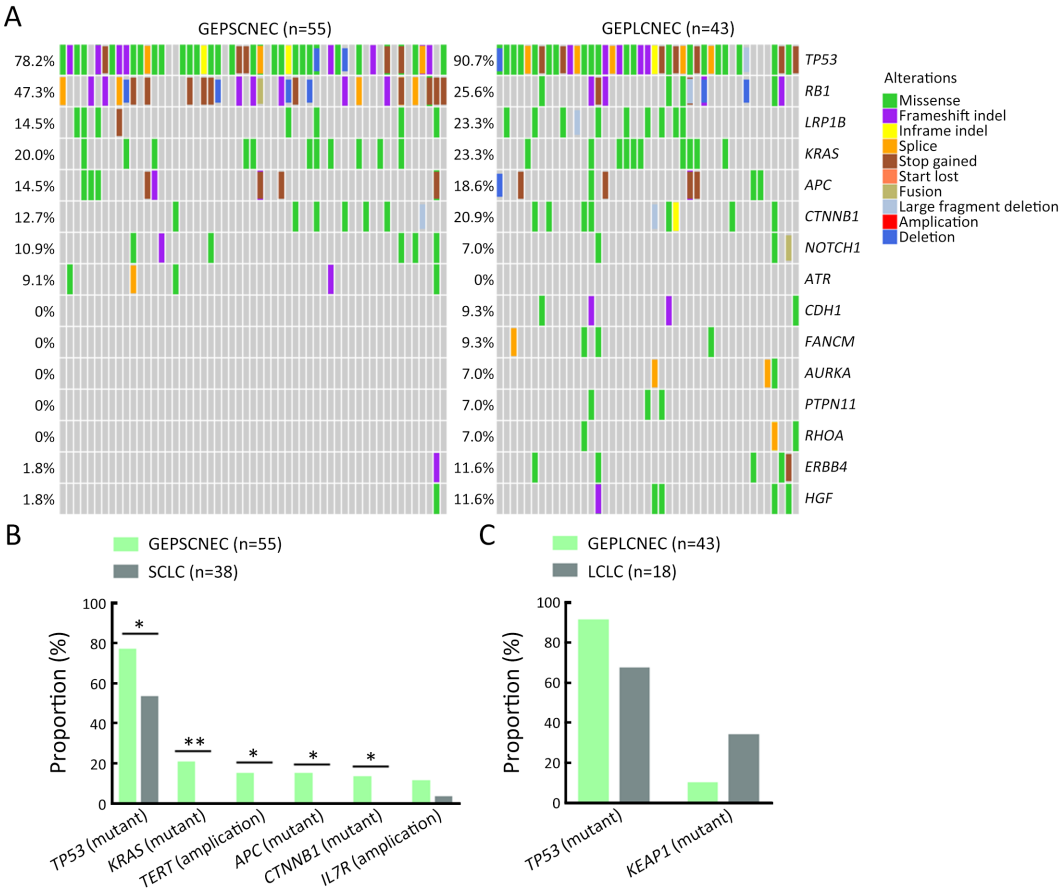

Figure S9 Summary of alteration frequency of GEPSCNEC *vs*. GEPLCNEC. (A) Oncoprints showing the top most frequent and significant gene alterations between GEPSCNEC and GEPLCNEC; (B) Bar plots of pathway alteration proportions of GEPSCNEC and SCLC; (C) Bar plots of pathway alteration proportions of GEPLCNEC and LCLC. GEPSCNEC, gastroenteropancreatic small-cell neuroendocrine carcinoma; GEPLCNEC, gastroenteropancreatic large-cell neuroendocrine carcinoma; SCLC, small-cell lung cancer; LCLC, large-cell lung cancer. P values were determined using Fisher's exact test. *, P<0.05; **, P<0.01.

Table S1 Comparison of clinical characteristics of GEPNEC and digestive adenocarcinoma

Characteristics	n (%)		P
	GEPNEC (N=99)	Digestive adenocarcinoma (N=101)	
Age (year) ($\bar{x}\pm s$)	59.6±12.2	60.3±11.7	0.532
Gender			0.451
Female	36 (36.4)	43 (42.6)	
Male	63 (63.6)	58 (57.4)	
Primary sites			–
Bile duct	8 (8.1)	7 (6.9)	
Colorectum	18 (18.2)	56 (55.4)	
Duodenum	9 (9.1)	–	
Esophagus	7 (7.1)	–	
Stomach	32 (32.3)	26 (25.7)	
Pancreas	12 (12.1)	12 (11.9)	
Unknown primary	13 (13.1)	–	
Pathology[#]			–
Undistinguishable	1 (1.0)	–	
LCNEC	43 (43.4)	–	
SCNEC	55 (55.6)	–	
Adenocarcinoma	–	101 (100)	
Mixed component			–
MiNEN	17 (17.2)	–	
Pure NEC	82 (82.8)	–	
Stage			<0.001
Stage I–III	17 (17.2)	61 (60.4)	
Stage IV	82 (82.8)	40 (39.6)	
Metastases			0.166
Liver	58 (58.6)	27 (26.7)	
Lung	12 (12.1)	7 (6.9)	
Bone	9 (9.1)	3 (3.0)	
NSE elevation			<0.001
No	34 (34.3)	72 (71.3)	
Yes	65 (65.7)	29 (28.7)	
Smoking			0.446
No	79 (79.8)	75 (74.3)	
Yes	20 (20.2)	26 (25.7)	

GEPNEC, gastroenteropancreatic neuroendocrine carcinoma; SCNEC, small-cell NEC; LCNEC, large-cell NEC; MiNEN, mixed neuroendocrine-non-neuroendocrine neoplasia; NSE, neuron-specific enolase. [#], one NEC patients had difficulty in identifying small or large cell subtypes.

Table S2 Summary of genomic characteristics of GEPNEC patients undergone target NGS

Year	Author (ref.)	N (n)*	Primary site (N)	N		%								
				LCNEC¶	Panel	*TP53*	*RB1*	*KRAS*	*BRAF*	*APC*	*CTNNB1*	*CDKN2A*	*PI3KCA*	*ERBB2*
2012	Karkouche R (1)	12 (8)	Colorectum	12	–	–	–	33	17	–	–	–	–	–
2017	Scardoni M (2)	6 (–)	Stomach	–	–	83	17	17	–	–	0	–	–	–
2015	Sahnane N (3)	89 (36)	Esophagus (6), stomach (36), duodenum (4), colorectum (37), gallbladder (3), pancreas (3)	40	–	–	–	17	7	–	–	–	–	–
2016	Olevian DC. (4)	29 (–)	Colorectum	–	–	–	–	17	59	–	–	–	–	–
2016	Bergsland EK (5)	123	Pancreas	–	192	18	10	7	–	3	–	21	–	–
2016	Bergsland EK (5)	92	Colon	–	192	59	34	37	–	47	–	5	–	–
2016	Bergsland EK (5)	59	Others: (esophagus, stomach, small intestine)	–	192	49	29	3	–	8	–	25	–	–
2016	Vijayvergia.N (6)	23 (4)	Colon (9), pancreas (4), small intestine (1), others (9)	–	50	57	9	30	13	22	–	–	22	–
2017	Makuuchi R (7)	6	Stomach	–	35	100	17	17	–	0	–	–	17	–
2017	Woischke C (8)	15 (10)	Colorectum	11	50	100	30	90	20	80	–	–	40	–
2018	Takizawa N (9)	25	Colorectum	16	–	21	–	8	–	4	4	–	–	–
2018	Moritz J (10)	19	Colorectum (MiNEN)	–	32	53	5	21	37	16	0	–	–	5
2018	Moritz J (10)	8	Colorectum (NEC)	7	32	63	0	25	25	63	0	–	–	0
2018	Konukiewitz B (11)	12	Pancreas	9	409	67	33	42	8	8	0	8	8	8
2019	Shamir ER (12)	25 (–)	Colorectum	–	479	38	58	63	–	63	–	–	–	–
2019	Chen LH (13)	83	Colorectum	–	–	66	17	37	20	60	–	–	–	–
2019	Dizdar L (14)	15	GEPNEC	–	–	–	–	–	47	–	–	–	–	–
2020	Krishnamurthy K (15)	2	GEPNEC	–	126	56	0	–	–	–	44	–	–	–
2020	Tanaka H (16)	44	Pancreas	17	–	–	55	49	–	–	–	–	–	–
2020	Busico A (17)	39§	GEP	–	50	59	–	3	10	8	5	–	–	–
2020	Puccini A (18)	135§	GEP	–	592	51	11	29	5	27	–	–	7	–
2021	Koh J (19)	13	Stomach (MiNEN)	12	170	69	0	0	8	31	8	–	–	–
2021	Koh J (19)	8	Stomach (NEC)	6	170	88	13	0	0	0	13	–	–	–
2021	Ishida S (MiNEN) (20)	6	Stomach	6	WES	67	0	0	–	33	17	–	–	–
2021	Ishida S (NEC) (20)	7	Stomach	4	WES	62	14	14	–	14	0	–	–	–
2021	Liu F (21)	15	Gallbladder	10	WES	73	27	–	0	0	27	–	–	0
2021	deBitter T (22)	9 (6)	Gallbladder	5	523	77	33	11	–	11	33	0	0	0
2022	Venizelos A (23)‖	181	GEP/Unknown	–	360	64	14	22	20	28	6	3	–	–
2021	Lee SM (24)	30	Colorectum	18	382	43	47	53	23	37	0	3	10	7

Table S2 (*continued*)

Table S2 (*continued*)

Year	Author (ref.)	N (n)*	Primary site (N)	N		%							
				LCNEC¶	Panel	*TP53*	*RB1*	*KRAS*	*BRAF*	*APC*	*CTNNB1*	*CDKN2A*	*PI3KCA*	*ERBB2*
2022	Yachida (25)	54	GEP			–	–	26	–	–	–	–	–	–
2022	Wu (26)	143	GEP		WES	89	25	8	–	18	–	–	–	–

GEPENC, gastroenteropancreatic neuroendocrine carcinoma; NGS, next-generation sequencing; MiNEN, mixed neuroendocrine-non-neuroendocrine neoplasia; LCNEC, large-cell NEC; WES, whole-exome sequencing. *, N means numbers of the total GEPNEC samples, n referred to the MiNEN mentioned in the cohort (if accessible) in the study; ¶, numbers of LCNEC patients; §, Puccini *et al.* and Busico *et al.* have not provided the relative proportions of NEC among the G3 NEN; ǀ, Venizelos *et al.* have included 29 G3 NET into the total 181 patients.

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Table S3 Comparisons of significant gene alteration frequency differences of intra-groups of GEPNEC patients

Gene	% (n/N)		OR (95% CI)	P
ENEC *vs.* LNEC				
TP53 (mutation)	100 (7/7)	56.1 (32/57)	0 (0–0.99)	0.036
SETD2 (mutation)	28.6 (2/7)	0 (0/57)	0 (0–0.60)	0.010
GNEC *vs.* LNEC				
TP53 (mutation)	87.5 (28/32)	56.1 (32/57)	0.18 (0.04–0.61)	0.002
RB1 (mutation)	28.1 (9/32)	49.1 (28/57)	2.55 (0.94–7.38)	0.033
NOTCH1 (mutation)	0 (0/32)	15.8 (9/57)	Inf (1.24–Inf)	0.024
TERT (amplification)	28.1 (9/32)	0 (0/57)	0 (0–0.24)	<0.001
IL7R (amplification)	21.9 (7/32)	0 (0/57)	0 (0–0.35)	<0.001
CCNE1 (amplification)	25.0 (8/32)	3.5 (2/57)	0.12 (0.01–0.64)	0.004
DNEC *vs.* LNEC				
TP53 (mutation)	100 (8/8)	56.1 (32/57)	0 (0–0.84)	0.019
KRAS (mutation)	37.5 (3/8)	3.5 (2/57)	0.07 (0.01–0.71)	0.011
IL7R (amplification)	25.0 (2/8)	0 (0/57)	0 (0–0.70)	0.013
TERT (amplification)	25.0 (2/8)	0 (0/57)	0 (0–0.70)	0.013
CRNEC *vs.* LNEC				
APC (mutation)	33.3 (6/18)	5.3 (3/57)	0.12 (0.02–0.63)	0.005
KRAS (mutation)	44.4 (8/18)	3.5 (2/57)	0.05 (0–0.29)	0.048
TP53 (mutation)	94.4 (17/18)	56.1 (32/57)	0.08 (0–0.56)	0.004
PNEC *vs.* LNEC				
RB1 (mutation)	16.7 (2/12)	49.1 (28/57)	0.21 (0.02–1.12)	0.039
KRAS (mutation)	25.0 (3/12)	3.5 (2/57)	9.02 (0.91–122.19)	0.031
BTNEC *vs.* LNEC				
ARID1A (mutation)	50.0 (4/8)	5.3 (3/57)	0.08 (0.01–0.79)	0.003
CTNNB1 (mutation)	37.5 (3/8)	3.5 (2/57)	0.05 (0–0.60)	0.011
TP53 (deletion)	25.0 (2/8)	0 (0/57)	0 (0–0.60)	0.013
FBXW7 (mutation)	37.5 (3/8)	1.8 (1/57)	0.03 (0–0.42)	0.005

GEPNEC, gastroenteropancreatic neuroendocrine carcinoma; OR, odds ratio; 95% CI, 95% confidence interval; ENEC, esophagus NEC; LNEC, lung NEC; GNEC, gastric NEC; DNEC, duodenum NEC; CRNEC, colorectal NEC; PNEC, pancreas NEC; BTNEC, bile tract NEC; Inf and –Inf, infinitive and –infinitive.

Table S4 Alterations frequency differences between SCNEC and LCNEC

Gene	% (n/N)		OR (95% CI)	P
SCNEC *vs.* LCNEC				
KEAP1 (mutation)	2.2 (2/93)	16.4 (10/61)	9.20 (1.86–89.54)	0.001
FANCM (mutation)	0 (0/93)	8.2 (5/61)	Inf (1.52–Inf)	0.008
TP53 (mutation)	66.7 (62/93)	83.6 (51/61)	2.83 (1.23–7.03)	0.011
CDH1 (mutation)	0 (0/93)	6.6 (4/61)	Inf (1.08–Inf)	0.021
KIT (mutation)	0 (0/93)	6.6 (4/61)	Inf (1.08–Inf)	0.021
RB1 (mutation)	51.6 (48/93)	31.1 (19/61)	0.47 (0.23–0.96)	0.025
CTNNB1 (mutation)	7.5 (7/93)	18.0 (11/61)	2.82 (0.93–9.13)	0.071
ERCC5 (mutation)	0 (0/93)	4.9 (3/61)	Inf (0.67–Inf)	0.055
FGFR1 (mutation)	0 (0/93)	4.9 (3/61)	Inf (0.67–Inf)	0.055
FLT1 (mutation)	0 (0/93)	4.9 (3/61)	Inf (0.67–Inf)	0.055
RHOA (mutation)	0 (0/93)	4.9 (3/61)	Inf (0.67–Inf)	0.055
ERBB4 (mutation)	2.2 (2/93)	9.8 (6/61)	5.14 (0.88–53.75)	0.058
NOTCH1 (mutation)	15.1 (14/93)	4.9 (3/61)	0.31 (0.05–1.18)	0.065
ATR (mutation)	8.6 (8/93)	1.6 (1/61)	0.15 (0–1.08)	0.088
GEPSCNEC *vs.* GEPLCNEC				
CDH1 (mutation)	0 (0/55)	9.3 (4/43)	Inf (0.87–Inf)	0.034
FANCM (mutation)	0 (0/55)	9.3 (4/43)	Inf (0.87–Inf)	0.034
ATR (mutation)	9.1 (5/55)	0 (0/43)	0 (0–1.35)	0.065
TP53 (mutation)	76.4 (42/55)	90.7 (39/43)	0.84 (0.23–1.12)	0.063

SCNEC, small-cell neuroendocrine carcinoma; LCNEC, large-cell neuroendocrine carcinoma; GEPSCNEC, gastroenteropancreatic SCNEC; GEPLCNEC, gastroenteropancreatic LCNEC; OR, odds ratio; 95% CI, 95% confidence interval; Inf and –Inf, infinitive and –infinitive.

Table S5 Alterations frequency differences between pure NEC and MiNEN

Gene	Pure NEC [% (n/N)]	MiNEN [% (n/N)]	OR (95% CI)	P
KRAS (mutation)	20.7 (17/82)	23.5 (4/17)	1.13 (0.30–5.13)	0.945
APC (mutation)	23.2 (19/82)	29.4 (5/17)	1.26 (0.20–2.97)	0.813
RB1 (mutation)	41.5 (34/82)	23.5 (4/17)	0.63 (0.63–10.4)	0.166
TP53 (mutation)	84.1 (69/82)	82.4 (14/17)	0.95 (0.18–4.96)	0.858

NEC, neuroendocrine carcinoma; MiNEN, mixed neuroendocrine-non-neuroendocrine neoplasia; OR, odds ratio; 95% CI, 95% confidence interval.

Table S6 Alterations frequency differences in GEPNEC and adenocarcinoma

Gene	% (n/N)		OR (95% CI)	P
GNEC *vs.* STAD				
ARID1A (mutation)	0 (0/32)	34.6 (9/26)	0 (0–0.21)	<0.001
TP53 (mutation)	84.4 (27/32)	57.7 (15/26)	4.20 (1.23–15.44)	0.014
PI3KCA (mutation)	0 (0/32)	15.4 (4/26)	0.01 (0–0.77)	0.016
LRP1B (mutation)	12.5 (4/32)	38.5 (10/26)	0.23 (0.06–0.84)	0.020
RB1 (mutation)	25.0 (8/32)	3.8 (1/26)	8.16 (1.08–369.00)	0.033
CRNEC *vs.* COREAD				
RB1 (mutation)	38.9 (7/18)	10.7 (6/56)	0.19 (0.05–0.67)	<0.001
PNEC *vs.* PAC				
KRAS (mutation)	16.7 (2/12)	75.0 (9/12)	0.10 (0.01–0.84)	0.034

GEPNEC, gastroenteropancreatic neuroendocrine carcinoma; GNEC, gastric NEC; STAD, stomach adenocarcinoma; CRNEC, colorectal NEC; COREAD, colorectal adenocarcinoma; PNEC, pancreas NEC; PAC, pancreatic adenocarcinoma.