Dissecting the genetic variations associated with response to first-line chemotherapy in patients with small cell lung cancer: a retrospective cohort study

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Background: Chemotherapy has been the standard treatment for small-cell lung cancer (SCLC) for decades. Nonetheless, patients are usually responsive to initial chemotherapy but quickly suffer from relapse, resulting in a poor long-term outcome. Treating advances that greatly ameliorate survival outcomes are historically finite, and credible biomarkers for therapeutic evaluation are deficient. As the genetic biology emerges, investigating biomarkers to optimize individualized treatment for SCLC is necessary.

Methods: Based on following inclusion criteria: (I) patients diagnosed as SCLC by pathology; (II) patients treated with first-line etoposide/cisplatin (EP) chemotherapy; (III) patients who received long-term follow-up and signed informed consent, a total of 24 SCLC patients receiving first-line standard chemotherapy were divided into progressive disease (PD) and partial response (PR) groups. They were regularly followed every 3 months with computed tomography (CT) scan until recurrences determined by CT scan results. Next-generation sequencing (NGS) with a panel of 1,406 cancer-related genes was conducted on the tumor tissue-derived DNA of patients to compare genetic variations, including deletions (indels), single nucleotide variations (SNVs), copy number variations (CNVs), and copy number instability (CNI) between the two groups.

Results: For the clinical characteristics of enrolled SCLC patients, except for significant differences in sex, age, clinical stage, and limited or extensive stage, PD patients showed distinctly shorter overall survival than those with PR (6.5 *vs.* 14.0 months, respectively, P=0.007). Genetic variations analysis discovered several common genes with CNV mutations between the PR and PD groups, and increased epidermal growth factor receptor (*EGFR*) gene copy numbers gain was found in PR groups in comparing with PD patients (P=0.006). However, no significant differences in terms of SNVs, indels, genotypes associated with first-line chemotherapy, CNI of tumor tissue-derived DNA, and tumor mutational burden of tumor tissues were observed between two groups. Additionally, the relationship between *EGFR* gene mutation and clinicopathological features of SCLC indicated that *EGFR* gene mutation may be an independent indicator for SCLC patients.

Conclusions: Increased *EGFR* gene CNVs may be an independent indicator influencing the survival time and PR in SCLC patients receiving standard first-line chemotherapy.

Keywords: Epidermal growth factor receptor (*EGFR*); first-line chemotherapy; genetic variation; small cell lung cancer (SCLC); somatic mutation

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Introduction

Lung cancer as the second most frequently diagnosed malignancy (1) is classified into non-small cell lung cancer (NSCLC) and small cell lung cancer (SCLC) in terms of the morphology of lung cancer cells (2). SCLC is an aggressive epithelial tumor showing a high susceptibility to early development and accounts for approximately 14% of all lung cancer cases (3). About two-thirds of SCLC patients also have metastasis (4). Currently, there is limited treatment options available for SCLC, suggesting that SCLC is one of the most fatal malignancies (5).

Regardless of the age, gender, or clinical stage of SCLC patients, chemotherapy is usually an essential part of treatment (6). In recent decades, etoposide and platinum-doublet chemotherapy has been applied as a standard therapy for SCLC, and combination chemotherapy with other agents, including irinotecan (Camptosar), carboplatin (Paraplatin), and DNA topoisomerase inhibitor, have been considered as standard second-line treatment (7-10). To ameliorate adverse reactions and drug resistance to chemotherapy alone, alternative therapeutic options such as radiotherapy, surgery, and/or immunotherapy are often introduced (4,11-14). Combined used of radiotherapy and chemotherapy could improve local control of patients with limited-stage SCLC and their overall survival. The most commonly used initial chemotherapy regimen for those with extensive-stage SCLC is etoposide/cisplatin (EP).

However, there is no chemotherapy combinations showing superior efficacy (15).

Because of recent advances in high-resolution detection technology, a new understanding of the genetic biology of SCLC has led to the development of more selective and targeted therapies, the most promising of which is that the genetic variability in individual patients may predict drug response and therapeutic efficacy or susceptibility to adverse drug reactions (16). At common RNA levels, an upregulated miR-27a expression after chemotherapy was seen in partial response (PR) patients than in those who exhibited no response (NR), and further survival analysis indicated that patients with reduced miR-27a levels displayed inferior outcomes than those with raised miR-27a levels (17). Furthermore, in epidermal growth factor receptor (EGFR)-mutant NSCLC patients, EGFR-tyrosine kinase inhibitors (TKIs) were adopted for considerable therapeutic effects (18). Genetic variation was also related to response to dutasteride for male undergoing androgenetic alopecia (19) as well as long-term therapeutic response in bipolar depression (20). What's more, the combination of genomic variation with other immunotherapy related indicators has been thought to be meaningful for precise immunotherapy decisions for advanced lung squamous cell carcinoma (21). All these findings highlight the importance of genetic variation in drug treatment. Nonetheless, similar studies on SCLC are rare (22,23). A few consistent associations have been reported for some individual susceptibility genes, but no general recommendations have been formulated to date (24-26).

Herein, this retrospective study aimed to investigate the clinical characteristics of genetic variations detected by single-nucleotide polymorphism (SNP) sequencing in tissue samples collected from SCLC patients prior to first-line chemotherapy. Then, the genotyping data of the patients were comprehensively analyzed between the progressive disease (PD) and PR groups. We present this article in accordance with the TRIPOD reporting checklist (available at https://jtd.amegroups.com/article/view/10.21037/jtd-23-1772/rc).

Highlight box

Key findings
- An increased epidermal growth factor receptor (*EGFR*) gene copy number may be a positive biomarker for small cell lung cancer (SCLC) patients treated with first-line standard chemotherapy.

What is known and what is new?
- EGFR mutations are found in a small number of SCLC patients, who may be sensitive to EGFR-tyrosine kinase inhibitors. There are few studies on the impact of prognosis in patients with EGFR copy number amplification.
- We compared the genetic biology of SCLC patients in partial response and progressive disease groups receiving first-line standard chemotherapy and found that EGFR amplification may be associated with treatment effects.

What is the implication, and what should change now?
- EGFR amplification may be a useful biomarker for chemotherapy-responsive patient selection, and further large-scale research should be conducted.

Methods

Patients and data collection

A total of 24 SCLC patients who underwent first-line standard chemotherapy (EP regimen) from October 2009 to February 2012 were enrolled in this retrospective study.

Table 1 General characteristics of study subjects in the two groups

Clinical features	EGFR gene copy number		P value
	Gain (n=8)	Non-gain (n=16)	
Gender, n (%)			0.718
Female	1 (12.5)	0	
Male	7 (87.5)	16 (100.0)	
Age			0.95
Mean (SD)	63.3 (5.63)	63.1 (8.74)	
Median [Min, Max]	62.5 [57.0, 70.0]	62.5 [48.0, 76.0]	
Stage, n (%)			>0.99
IIb–IIIa	2 (25.0)	3 (18.8)	
IIIb–IV	6 (75.0)	13 (81.3)	
Limited or extensive stage, n (%)			0.884
Extensive stage	4 (50.0)	10 (62.5)	
Limited stage	4 (50.0)	6 (37.5)	

EGFR, epidermal growth factor receptor; SD, standard deviation.

For enrolled patients, they were regularly followed every 3 months with computed tomography (CT) scan until recurrences determined by CT scan results. They were allocated into a PD group (n=10) or a PR group (n=14) based on the curative effect of chemotherapy assessed by CT scan results. PD was defined as the appearance of new lesions or a >25% increase in the size of lesions, while PR was defined as a >50% reduction in the size of the lesions. The inclusion criteria were as follows: (I) patients diagnosed as SCLC by pathology; (II) patients treated with first-line EP chemotherapy; (III) patients who received long-term follow-up and signed informed consent. Patients were excluded based on the following criteria: (I) history of other malignancies; (II) history of myocardial infarction, unstable angina pectoris, stroke, or uncontrollable arrhythmias; (III) pregnant or lactating patients; (IV) history of mental illness; (V) poor compliance. The clinical characteristics of SCLC patients are summarized in *Table 1*.

The sample size of the trial was determined by the analysis of overall survival. We calculated that 26 deaths in the chemotherapy treated SCLC population would be needed to provide 90% power at a two-sided significance level of 0.05 to detect a significance between treatment-resistance and treatment-sensitive group.

Tumor specimens were acquired by surgery (>2% of total tissue mass and >150 cells). Diagnosis of SCLC was confirmed by pathologists using Formalin-fixed and paraffin-embedded tissues. Tumor-node-metastasis (TNM) staging system of International Association for the Study of Lung Cancer (version 7) was used to determine the clinical staging. Chi-squared test or Fisher's exact test were analyzed categorical variables for baseline comparability.

The study was approved by the Ethics committee of the First Affiliated Hospital of Guangzhou Medical University (No. 202015). Written informed consent was signed by all the patients. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013).

DNA extraction

Tissue samples were collected from SCLC patients prior to first-line chemotherapy and embedded in paraffin (5–10 μm thick for each section). Pathological examination was performed to calculate the quantity of tumor content, which was required to be no less than 10%. Then, NovaSeq S-Prime Reagent Kits (Illumina, California, USA) was employed to extract DNA from the paraffin-embedded tissue blocks. DNA concentration and quality was assessed using Qubit dsDNA HS (High Sensitivity) Assay Kit (Thermo Fisher, Waltham, USA) and Agilent 2100 BioAnalyzer (Agilent, Palo Alto, USA), respectively.

Library preparation and next-generation sequencing (NGS)

A total of 74.5 ng DNA per tissue sample was isolated to construct the DNA libraries. Firstly, genomic DNA was sheared into 160–200 bp small fragments by the Covaris LE220 (Covaris, New Jersey, USA). Subsequently, a KAPA Hyper Preparation Kit (Kapa Biosystems, Boston, USA) was used to construct a fragmented DNA library. The libraries were quantified with an AccuGreen High Sensitivity dsDNA Quantitation Kit (Biotium, California, USA), and their size was determined by an Agilent Bioanalyzer 2100 (Agilent). The DNA library was captured by a designed panel of 1,406 cancer-related genes (Genecast Biotechnology Co. Ltd., Beijing, China) that are frequently mutated in common solid tumors and span a 2.4 Mb region in the human genome. Finally, the captured library was subjected to NovaSeq 6000 (Illumina) sequencing, which produced paired-end sequencing with the length of each end as 150 bp.

Sequencing data analysis

After obtaining the NGS sequencing and confirming quality control, the genomic loci were examined and mapped to the human genome reference (HG19) with a Burrows-Wheeler Aligner (BWA, v0.7.17) (27). Subsequently, the differences in single nucleotide variations (SNVs) and deletion (indels) were determined using VarScan2 (RRID: SCR_006849) and the algorithm independently developed by Genecast Biotechnology Company. SNV is mainly based on the pileup of sequencing reads obtained, and the mutation type and frequency can be inferred according to the number of different supporting bases in the same genome location and sequencing quality. To determine the presence of copy number variations (CNVs), CONTRA software (version 2.0.8) was used to call copy number values from the tumor tissue-derived DNA of patients by sequencing depth and comparison with a healthy population baseline, with a copy number threshold of 3 for CNV gain and 1.2 for CNV loss. Copy number instability (CNI) was defined as the sum of the fluctuation levels of all gene copy numbers in the panel relative to 30 normal people randomly selected from the normal population database of Genecast Biotechnology Company. For CNI calculation, the copy numbers were called after mapping using the BWA tool. After applying proprietary algorithms for tumor DNA sequencing to correct for GC-content and mappability, the read counts

were converted into log2 ratios and Z-values based on Gaussian transformations compared with 30 normal people. Target areas with a Z-score >95th percentile and twice the absolute standard deviation of the normal control group were retained, and CNI was the sum of these Z-scores.

Calculation of tumor mutational burden (TMB)

TMB refers to the total number of somatic missense mutations in a baseline tumor sample. TMB was calculated based on the number of somatic nonsynonymous SNVs (depth >100× and allele frequency ≥0.05) detected on NGS (interrogating Mb of the genome), followed by extrapolation to the whole exome using the validated algorithm. Alterations that were likely or known to be bona fide oncogenic drivers were excluded. TMB was calculated in mutations per Mb.

Statistical analyses

SPSS version 20.00 was used for the statistical analysis. Fisher's exact test, nonparametric log-rank test, and Wilcoxon rank-sum test were used to determine the differences in somatic mutations, CNV, TMB, and CNI between the PD and PR groups. The comparison of overall survival between the two groups was analyzed by the Mann-Whitney U test. A statistically significant difference was defined when P value <0.05.

Results

Clinical characteristics of SCLC patients

The median age of patients in the PD and PR groups was 62 and 64.5 years, respectively (*Table 1*). Sex, age, clinical stage, and limited or extensive stage showed no significant differences (all P>0.05) between the PD and PR groups. PD patients showed significantly lower median overall survival than those with PR (6.5 *vs.* 14.0 months, respectively, P=0.007) (*Figure 1*).

CNVs in SCLC tissue DNA

As seen in *Figure 2A*, the incidence of CNV in the PR group was higher than that in the PD group, and there were nine common genes with CNVs in both groups, including *FDPS*, *MAP2K2*, *PSMA8*, *MALAT1*, *DDX27*, *GSTT1*, *SDHA*, *SMAD2*, and *VEGFA*. Compared with the PD

group, increased gene copy numbers of *EGFR* and *SQX2-OT* were only observed in the PR group (P=0.006 and P=0.053, respectively). Additionally, 21% of patients had an increased *SQX2-OT* gene copy number gain, relatively close to the 27% detected in our previous study (28). Except for *EGFR*, CNVs in other genes showed no obvious difference between the two groups using Fisher's exact test (all P>0.05). Additionally, we also analyzed the relationship between EGFR gene mutation and clinicopathological features of SCLC. Surprisingly, we found that EGFR gene mutation had no obvious connections with clinicopathological features in SCLC patients (*Table 2*). This observation

indicated that EGFR gene mutation may be an independent indicator for SCLC patients.

Comparison of somatic mutations between the PD and PR groups

The related genes and SNP sites of chemotherapy drugs for SCLC recommended by the National Comprehensive Cancer Network (NCCN) guidelines in PharmGKB (https://www.pharmgkb.org/) (29) were included in our study. A tumor tissue-derived DNA single nucleotide mutation map before first-line EP treatment showed that the *TP53* gene mutation was detected in four cases in the PD group and three cases in the PR group, with no significant difference (P=0.393) (*Figure 2B*). In addition, genes of nonsynonymous SNV (*BCL2*, *SOX2*, and *ZFHX3*), non-frameshift deletion (*FGFR1*), and stopgain (*CYP2C19*) were found in the PD group, while genes of nonsynonymous SNV (*ASPM*, *CDKN2A*, *CREBBP*, *EGFR*, *NOTCH2*, *NRAS*, *PTPRB*, *RB1*, and *ROS1*) and non-frameshift deletion (FGFR1) were identified in the PR group (all P>0.05).
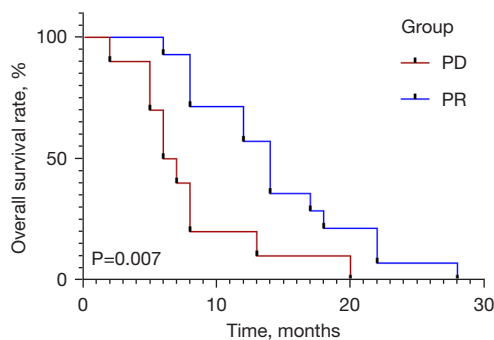
Analysis of chemotherapy-related gene loci in tumor tissue-derived DNA

To analyze the differences in the genotype of chemotherapy-

Figure 1 The overall survival of SCLC patients. PD, progressive disease; PR, partial response; SCLC, small cell lung cancer.
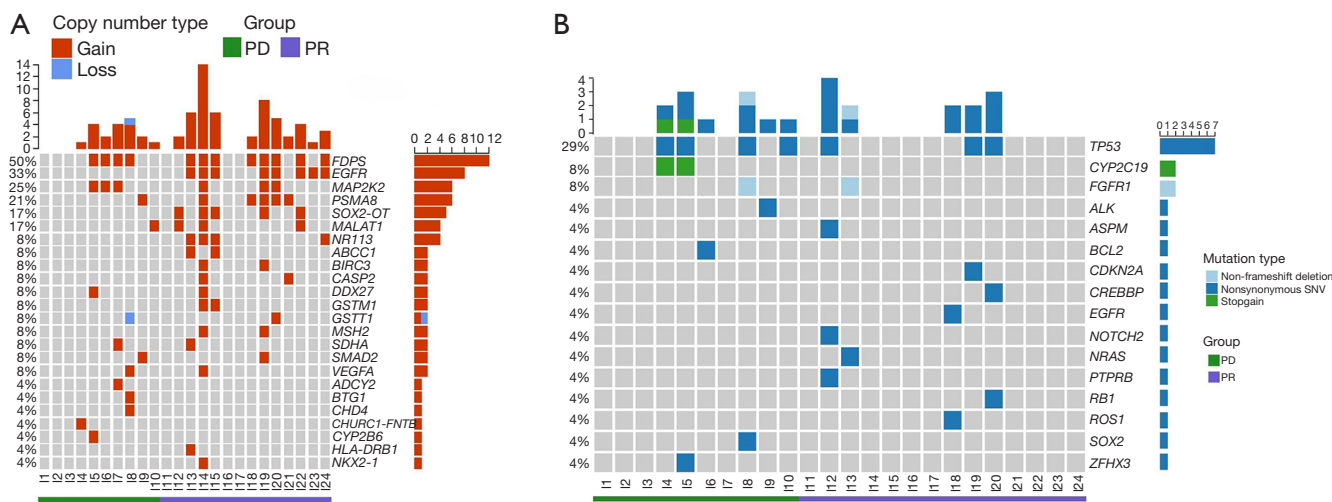

Figure 2 Comparison of genetic variations between the PD and PR groups. (A) Tile plots showing copy number variations of 24 genes in the two groups. (B) Tile plots showing known somatic mutations of 16 genes in the two groups. The X-axis represents the number of patients; the y-axis represents mutant genes and the frequency of gene mutations. The top bar (on the right) represents the number of mutations a patient carried. The P value is based on Fisher's exact test. PD, progressive disease; PR, partial response; SNV, single nucleotide variation.

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Table 2 The relationship between *EGFR* gene mutation and clinicopathological features in SCLC

Characteristics	Progressive disease group (n=10)	Partial remission group (n=14)	P value
Male	10 (100.0)	13 (92.9)	>0.99
Age (years)	62.00 [48.00, 76.00]	64.50 [49.00, 70.00]	0.827
Clinical stage			0.441
IIb–IIIa	2 (20.0)	3 (21.4)	
IIIb–IV	8 (80.0)	11 (78.6)	
Limited or extensive stage			0.4212
Limited stage	3 (30.0)	7 (50.0)	
Extensive stage	7 (70.0)	7 (50.0)	
Overall survival (months)	6.50 [5.00, 9.25]	14.00 [8.00, 19.00]	0.007

Data are presented as median [interquartile range] or n (%). *EGFR*, epidermal growth factor receptor; SCLC, small cell lung cancer.

related gene loci in tumor tissue-derived DNA between the PD and PR groups, we selected six genotypes related to first-line treatment efficacy (DYNC2H1_rs716274, ERCC1_rs11615, XRCC1_rs25487, GSTP1_rs1695, XPC_rs2228001, and SLIT1_rs2784917). The correlation between chemotherapy efficacy and the six genotypes was analyzed with the log-rank test. Results showed no significant difference in the genotype of chemotherapy-related gene loci between the two groups (all P>0.05) (*Figure 3*).

Analysis of TMB and CNI

As shown in *Figure 4A*, the TMB of tumor tissues from SCLC patients in the PR group was higher than in the PD group, but there was no significant difference (P=0.102). Likewise, no significant difference was observed in the CNI of tissue DNA between the two groups (P=0.505) (*Figure 4B*).

Discussion

SCLC is a metastatic malignancy with high recurrence and poor prognosis. Of the 24 patients recruited in this study, more than half had extensive-stage SCLC, suggesting that the cancer had spread to other organs, such as the other lung, bone, brain, or bone marrow. This retrospective analysis aimed to establish a landscape of genetic variations in Chinese SCLC patients receiving standard first-line chemotherapy and to correlate these variation profiles with clinical outcomes, including remission status and survival time. Our results demonstrated significant differences in the CNVs of increased *EGFR* gene copy numbers and overall survival time between patients who achieved PR after first-line chemotherapy and patients who developed PD.

In China, the commonly mutated genes in SCLC include *TP53*, *RB1*, *LRP1B*, and *PTEN* (30). In addition, a study by Xu *et al.* found that *RB1* mutations were found in 83.6% of patients with SCLC in China (31). In our study, NGS with a designed panel was performed on tumor tissue-derived DNA to identify the gene mutations related to SCLC. We found that the commonly mutated genes in SCLC patients in the PD and PR groups were nonsynonymous SNV of *TP53* and indels of *FGFR1*, with no significant difference. Yokouchi *et al.* (32) have pointed out that the nonsynonymous somatic *TP53* mutation in SCLC patients is an independent factor for prolonged relapse-free survival rather than overall survival. Nevertheless, no significant differences in SNV of *TP53* and indels of *FGFR1* were found between the two groups.

It has been reported that widespread somatic CNVs included *MYC* amplification, copy number gains of *SOX2* at 3q26, *CCNE1* at 19q12, *FGF10* at 5p13, and *SOX2* at 3q26, and copy number losses of *FHIT* at 3p14, *PTEN* at 10q23, *RASSF1* at 3p21.3, *TP53* at 17p13, and *RB1* at 13q14.2 (28). Our data showed that the common genes involved in CNVs included *FDPS*, *EGFR*, *MAP2K2*, *MALAT1*, *DDX27*, *GSTT1*, *SDHA*, *SMAD2*, and *VEGFA*, which was partially different from our previous study. However, we repeatedly checked the data of the detection results and found no *RB1* alteration or *MYC* amplification, which could be explained by the small sample size. Further group comparison showed a statistically significant difference in the increased *EGFR* gene copy number between the PD and PR groups (P<0.05).
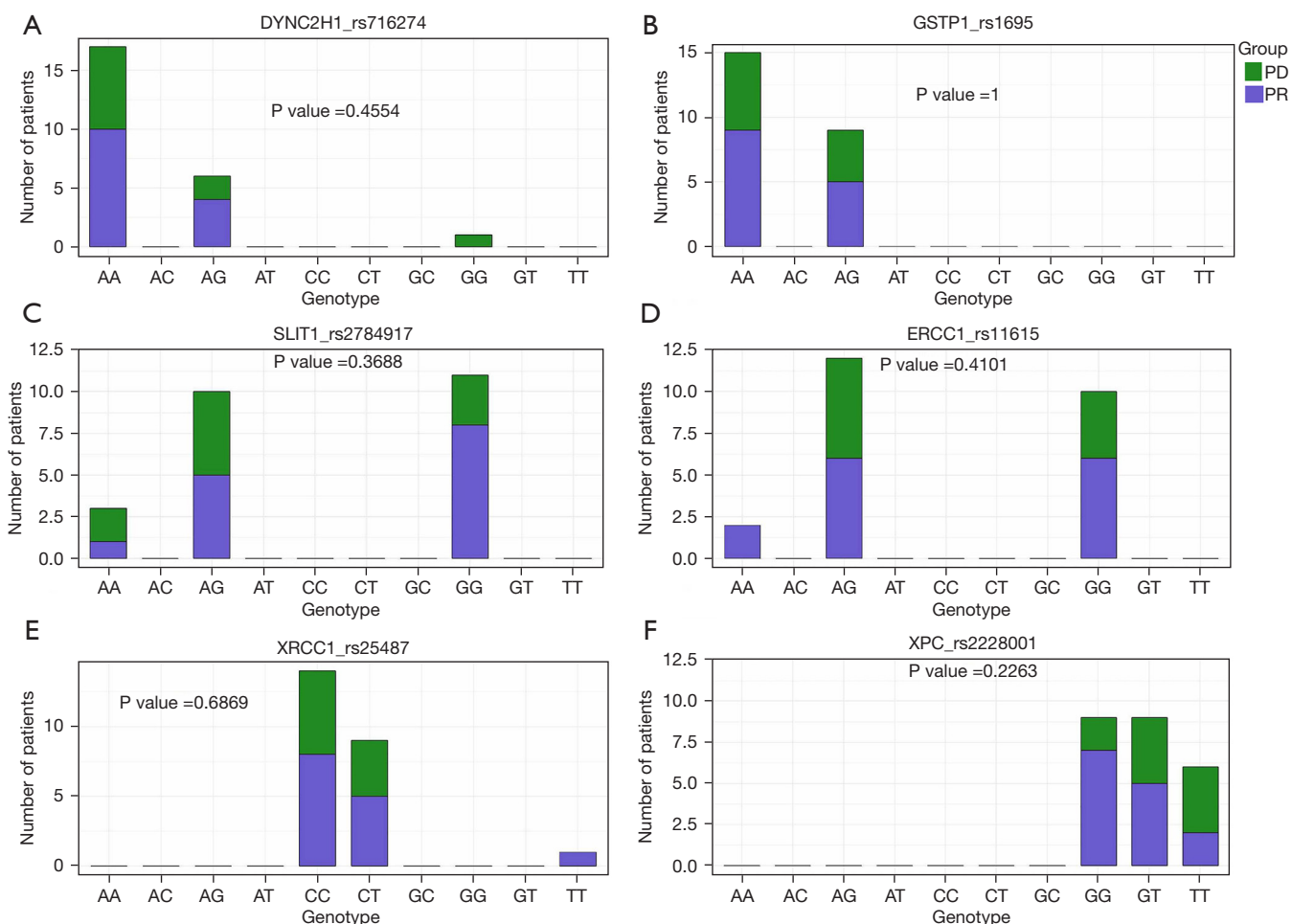
Figure 3 Analysis of genotypes that influence chemotherapeutic susceptibility in the PD and PR groups. (A-F) The color-coded bar graph shows the genotype distribution of six gene loci with two alleles in the PD and PR groups. The X-axis represents the genotype of chemotherapy-related gene loci; the y-axis represents the number of patients. DYNC2H1_rs716274, GSTP1_rs1695, SLIT1_rs2784917, ERCC1_rs11615 variant genotypes: homozygote (AA), heterozygote (AG), and homozygote (GG); XRCC1_rs25487 genotype: homozygote [1], heterozygote (CT), and homozygote (TT); XPC_rs2228001 genotype: homozygote (GG), heterozygote [2], and homozygote (TT). The P value is based on the nonparametric log-rank test. PD, progressive disease; PR, partial response.
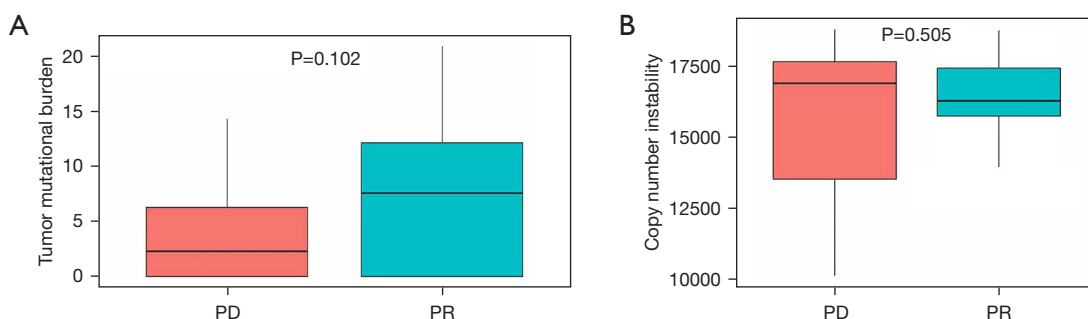

Figure 4 Comparison of tumor mutational burden and copy number instability between the PD and PR groups. (A) The color-coded bar graph of tumor mutational burden values in the two groups. (B) The color-coded bar graph of copy number instability values in the two groups. The P value is based on the nonparametric Wilcoxon rank-sum test. PD, progressive disease; PR, partial response.

EGFR is a member of the erbB family of tyrosine kinase receptors, and the *EGFR* gene coding the receptor is localized at chromosome 7 (33). The normal physiological function of *EGFR* is to regulate epithelial tissue development and homeostasis. However, under pathological environment, such as lung, breast cancer as well as glioblastoma, it was demonstrated to be a driver of tumorigenesis (34). As research deepens, abnormally activated EGFR in tumor mainly accounts for amplification and point mutations at the genomic locus, which lead to unfavorable survival (35,36) and tolerance to various chemotherapy drugs (37). These findings drive the predicted value of EGFR gene copy number. Therefore, given that *EGFR* mutations are detected in up to 50% of NSCLC, *EGFR* has also become a critical target in NSCLC treatment (38). Higaki *et al.* further observed that patients with *EGFR* gene copy number gain but not amplification, including those exhibiting polysomy, also exhibited poorer prognosis than gene copy number non-gain patients. This study indicated that the higher mutation status may result in greater drug tolerance (36). In contrast, *EGFR* gene mutations are rare in SCLC, accounting for only 2.6–7.1% of SCLC patients in China (39). In 2006, Okamoto *et al.* were the first to report an *EGFR* mutation (heterozygous in-frame 15-base pair deletion) in a gefitinib-responsive SCLC patient (40). Two years later, Tatematsu *et al.* examined the *EGFR* gene copy number in five SCLC patients with *EGFR* mutations and found gene amplification in four cases (41). Since then, some SCLC cases with *EGFR* mutations have been reported successively, showing that *EGFR* mutations are sensitive to EGFR-TKIs and may suggest a positive prognostic efficacy (42-44). Conversely, one prior report has clarified that *EGFR* is low expressed in SCLC, suggesting that EGFR-TKIs are ineffective against SCLC even when *EGFR* is mutated (41).

Despite its rapid growth and early metastasis, SCLC is more chemosensitive and radiosensitive than other lung cancers. Chemotherapy containing EP has been the standard therapy for extensive-stage SCLC for decades (6). Kim *et al.* have clarified patients treated with EP regimen showed an average rate of response of 60% to 80%, with a median overall survival of 8 to 10 months (45). This study demonstrated a noticeably better survival of patients in the PR group than in the PD group (14 *vs.* 6.5 months). As mentioned, PR was achieved in eight SCLC patients with an increased *EGFR* gene copy number who were treated with the EP regimen. Taken together, the longer overall survival in the PR group indicated a positive therapeutic effect of standard first-line chemotherapy in Chinese SCLC patients, which might be related to the increased *EGFR* gene copy number.

To determine the differences in the genotype of chemotherapy-related gene loci between the PD and PR groups, six genotypes related to first-line treatment efficacy, including *DYNC2H1_rs716274*, *ERCC1_rs11615*, *XRCC1_ rs25487*, *GSTP1_rs1695*, *XPC_rs2228001*, and *SLIT1_ rs2784917* were selected. Results showed no significant difference in the genotypes of chemotherapy-related gene loci between the two groups. However, one report that analyzed the association and multiple interactions of five *XRCC1* polymorphic variants in regulating lung cancer risk in a North Indian population described a positive correlation between *XRCC1 Gln632Gln* and lung cancer, whereas *Arg399Gln*, *XRCC1*, and *Arg194Trp* had no protective effect (46). In addition, in the present study, the TMB between the PD and PR groups was not significantly different. Likewise, no significantly different CNI value in the tumor tissue-derived DNA of SCLC patients was noticed between the two groups.

Conclusions

In summary, SCLC patients in the PR group showed higher overall survival after first-line chemotherapy (EP regimen) treatment, which may be associated with the increased *EGFR* gene copy number. This study has some limitations, such as the lack of immunohistochemical analysis and constitutional genetic information for the SCLC patients, as well as the limited sample size. Tissue DNA from only 24 cases may have caused less tumor cells to be tested, which may be the reason for the fewer gene mutations detected. In addition, the lack of a control group may have led to an inability to accurately filter out germ-line mutations, which may have affected the analysis results. Patients in this study ranged from stages II to IV, but the molecular typing of patients at different stages differs, which requires further study based on a fixed stage of SCLC patients in the future.

Acknowledgments

Footnote

Reporting Checklist: The authors have completed the TRIPOD reporting checklist. Available at https://jtd.

amegroups.com/article/view/10.21037/jtd-23-1772/rc

Data Sharing Statement: Available at https://jtd.amegroups.com/article/view/10.21037/jtd-23-1772/dss

Peer Review File: Available at https://jtd.amegroups.com/article/view/10.21037/jtd-23-1772/prf

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at https://jtd.amegroups.com/article/view/10.21037/jtd-23-1772/coif). H.Z. is from Genecast Biotechnology Co. Ltd., Wuxi, Jiangsu, China. J.Z. is from HaploX Biotechnology, Shenzhen, Guangdong, China. The other authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study was approved by the Ethics committee of the First Affiliated Hospital of Guangzhou Medical University (No. 202015). Written informed consent was signed by all the patients. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013).

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