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ORIGINAL ARTICLE

Genomic and Mutational Alterations of Lung Cancer Tissues and Pleural Effusion Samples in Zhoushan Archipelago, China

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ABSTRACT

Background: **This study aimed to characterize the genetic features of lung cancer tissues and pleural effusion samples from advanced Zhoushan Archipelago patients, providing insights for genome-guided precision therapy.**
Methods: **Genomic profiling was conducted by collecting 12 tissues and 12 pleural effusion samples. Whole-exosome sequencing was applied to the tissue samples, while targeted gene sequencing was performed on the effusion samples using next-generation sequencing technology.**
Results: **A total of 89.86% of the mutations in the tumor tissues from the Zhoushan cohort were single nucleotide polymorphisms (SNPs), with C > T being the most dominant single nucleotide variation (SNV) class. The top 10 mutated genes included *EGFR*, *MUC4*, *MUC18*, *TP53*, *ABCC4*, *ADGRV1*, *AGXT*, *LRRC4C*, *MAGEL2*, and *OR1F1*. In addition, 54.05% of the mutations in the pleural effusion samples exhibited SNPs, with C > T being the most dominant SNV class. The top 10 mutated genes comprised *SETD8*, *KMT2C*, *ANKRD11*, *ARID5B*, *PIK3CA*, *FCGR3A*, *RYR2*, *MED12*, *RNF43*, and *H3F3A*. The tumor tissues had a notably higher incidence of missense mutations and a lower rate of frame-shift deletions compared to the pleural effusion samples.**
Conclusions: **We identified the genomic profiles of lung cancer tissues and pleural effusion samples in Zhoushan Archipelago, providing valuable information for genome-guided precision therapy.**
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INTRODUCTION

Lung cancer ranks first in global malignant tumor mortality. In China, according to recent cancer data, the incidence and mortality rates of lung cancer have escalated, posing a serious threat to residents' health [1]. The main histologic subtype of lung cancer is lung adenocarcinoma, which constitutes nearly half of all cases [2]. Previous studies have identified mutated genes in Chinese lung adenocarcinoma patients, including *EGFR*, *KRAS*, *ALK*, *ERBB2*, *LRP1B*, *RET*, and others [3]. In addition, Chang and colleagues have reported

TP53, *KRAS*, *EGFR*, *NF1*, *BRAF*, *MET*, and *RIT* as the most common mutated genes [4]. The gene mutation distribution varies across Asian populations [5]. Zhoushan Archipelago, which is situated in eastern China, has hosted residents on various islands for generations. However, the genomic profiles of lung adenocarcinoma patients in Zhoushan Archipelago remain unclear [4,6]. The lack of clarity in the genetic landscape underscores the necessity for further research to understand the unique genomic characteristics of lung adenocarcinoma in this specific region.

Recent advancements in next-generation sequencing technology and precision therapy targeting driver gene mutations have significantly improved the therapeutic outcomes and overall prognosis for lung cancer patients [7,8]. Despite these advances, a considerable number of patients miss optimal surgical opportunities until tumor detection, hindering access to tumor tissue for subsequent sequencing and targeted therapy. As a solution, various sample types, including peripheral blood and pleural effusion, have been identified to assess the tumor tissue status in advanced lung cancer patients. Compared to peripheral blood, the gene landscape of pleural effusion samples closely mirrors that of tumor tissue. Detecting gene mutations in pleural effusion samples has proven valuable in guiding lung cancer treatment [9,10]. Furthermore, understanding gene mutations in advanced lung cancer patients from Zhoushan Archipelago and exploring the disparities between tumor tissues and pleural effusion samples add an intriguing dimension to this study. This information will contribute to a comprehensive understanding of the genetic aspects of lung cancer in this specific population and will aid in refining treatment strategies. In this study, we conducted whole-exon sequencing on 12 lung cancer tissues and targeted gene sequencing on 12 pleural effusion samples from advanced lung cancer patients in Zhoushan Archipelago. A comparative analysis with the Cancer Genome Atlas (TCGA) database provides a broader perspective, contributing vital information for genome-guided precision therapy in Zhoushan Archipelago, China.

MATERIALS AND METHODS

Patients

Fresh frozen tumor tissues and matched normal adjacent tissues were obtained from 12 lung cancer patients who underwent surgical resection at Zhoushan Hospital between March 2014 and August 2017. The diagnosis of lung cancer was confirmed by senior pathologists based on the World Health Organization criteria. Genetic profiling was performed by Annoroad Gene Technology. The tumor and adjacent tissue samples were genetically profiled by Annoroad Gene Technology (Beijing) using standard protocols. Twelve pleural effusion samples were collected from lung cancer patients treated at Zhoushan Hospital between March 2020 and March 2022. The pleural effusion (15 mL) was extracted from each patient, centrifuged at 3,000 rpm for 10 min, and the supernatants were stored at -80°C until detection. The genetic profiling of the pleural effusion samples was performed by MultiSciences Biotech Co., Ltd., according to standard protocols.

The demographic and pathologic data for all patients were obtained from their electronic medical records. Patients were the indigenous inhabitants of Zhoushan Archipelago were included. The study protocol, including sample collection, was reviewed and approved by the Ethics Review Committee of Zhoushan Hospital (2024-011), with written consent obtained from each participant.

Whole-exon sequencing of tumor tissues

The genomic DNA of fresh lung cancer tissues and adjacent normal lung tissues was isolated using a TIANamp Genomic DNA Kit (Tiangen, Beijing, China) according to the manufacturer's instructions. The tumor cellularity was confirmed by histopathological assessment of H&E-stained sections with all cases exceeding 40%. The concentration and purity of the isolated DNA were assessed using a Nanodrop spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA), a Qubit 3.0 Fluorometer (Life Technology, Carlsbad, CA, USA), and 1% agarose electrophoresis.

Subsequently, 200 ng of genomic DNA underwent fragmentation, repair, and ligation of paired-end adaptors. The purified DNA with Agencourt AMPure XP magnetic beads was amplified for the adaptor-ligated library. The quality of the library was checked, and it was then hybridized for 16 h at 65°C, binding to the capture beads. Following bead washing, post-capture amplification was conducted with indexing primers. Library quantification was assessed using a Qubit 3.0 Fluorometer (Life Technology) and an Agilent 2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA, USA). The resulting DNA libraries were sequenced using paired-end 150 bp on the high-throughput Illumina Hiseq xten platform (Illumina Inc., San Diego, CA, USA).

Targeted gene sequencing of pleural effusion samples

The supernatant of the pleural effusion samples was separated by centrifugation at 2,000 × g for 10 minutes within 1 day of collecting the samples to remove the cell debris and prevent the DNA degradation. The genomic DNA of the pleural effusion was purified using a plasma-free DNA isolation kit (Concert, Xiamen, China). A liquid-phase chip capture system was employed to enrich the target region DNA of the pleural effusion sample, followed by high-throughput sequencing on a NovaSeq 6000 sequencer (Illumina Inc., San Diego, CA, USA). Briefly, the quantity and quality of DNA were assessed using a Nanodrop spectrophotometer, Qubit 3.0 fluorometer, and 1.0% agarose electrophoresis. The qualified DNA for library preparation was randomly broken into DNA fragments of 150 - 300 bp in

length. The fragmented DNA underwent end repair, A-tailing at the 3′ end, and ligation with double-end adapters to construct a genomic DNA library. The library was subjected to liquid-phase hybridization with biotin-labeled exon probes, captured by streptomycin magnetic beads, and underwent quality inspection after polymerase chain reaction amplification. A NovaSeq 6000 sequencer (Illumina) was utilized for paired-end 150 sequencing. Targeted sequencing involved the detection of 611 genes using the Target Capture Array from Agilent Technologies, as identified from the data.

Data processing

The original data underwent filtering using fastp software, followed by mapping to the human reference genome GRCh37 with BWA software. Removal of duplicates was performed based on the comparison results. GATK Mutec2 software was used to calibrate the quality values, ensuring stability and reliability of the sequencing quality. Annovar software was adopted to annotate the mutated genes, and VARSCAN2 software (http://varscan.sourceforge.net/) was employed to analyze single nucleotide variations (SNVs) and insertions/deletions (indel). The tumor mutation burden (TMB) was defined as the total number of non-synonymous SNV, and indel per mega-base (1 Mb) of sequenced coding genomic regions. Open data of Asian lung cancer were screened from the TCGA database, with a total of 23 files selected. Subsequently, the targeted sequencing data, whole-genome sequencing data of tumor tissues, and TCGA data were subjected to comprehensive analysis.

Statistical analysis

All statistical analyses were performed using SPSS 22.0 (IBM SPSS Statistics for Windows; IBM Corp., Armonk, NY, USA) or GraphPad Prism 9.0 (GraphPad Software, La Jolla, CA, USA). The Mann-Whitney test or *t*-test was employed to analyze the difference in mutation status between the tumor tissues and pleural effusion samples, considering the normal distribution of the data. p-values ≤ 0.05 were deemed statistically significant with a two-sided test.

RESULTS

Patient characteristics

The demographic and clinical data of the 12 patients included in the tumor tissue sequencing cohort are presented in Supplementary Table 1. The median age of the cohort was 56.5 years old (range: 35 - 69 years old), with 5 male and 7 female participants. All patients had lung adenocarcinoma, with four showing lymph node metastases. Pathological staging revealed seven patients in stage IA, one patient in stage IB, three patients in stage IIB, and one patient in IIIA. For the cohort undergoing pleural effusion sequencing (Supplementary Table 2), the median age was 70.0 years old (range: 56 - 87 years old), including seven males and five females. Among them, tumor cells were detected in six patients. The distribution of the lung cancer subtypes included one patient with small cell lung cancer, nine patients with lung adenocarcinoma, and two patients with squamous cell lung cancer. Clinical staging indicated three patients in stage IIIB and four patients in stage IVA.

Mutation status of tumor tissues from lung cancer patients

The reads generated from whole-exome sequencing (WES) of 12 lung cancer samples were aligned to the human reference genome (GRCh19). The average mapping rate, on-target rate, and duplication rate were 99.95%, 45.68%, and 22.24%, respectively. The average sequencing depth across the targeted exome was 100×, with a mean coverage depth of 112.58×. Additionally, 97.28% of the targeted bases were covered at a depth of at least 10×. Reliable somatic mutations were identified after excluding germline variants detected in paired adjacent lung tissues. In the tissue cohort, a total of 897 mutations were identified in 795 genes, encompassing 38 frame-shift deletions, 25 frame shift insertions, 26 in-frame deletions, 759 missense mutations, 47 nonsense mutations, and 2 nonstop mutations (Figure 1A).

According to the mutation classification, single nucleotide polymorphisms (SNPs) accounted for 89.86% (806/897), insertions accounted for 2.79% (25/897), and deletions accounted for 7.139% (64/897, Figure 1A). The dominant SNV class was C > T, accounting for 33.42% (270/808), followed by C > A (23.76%, 192/808, Figure 1A). The details of the mutation status of each sample are presented in Table 1. The mean and median values for mutation counts and tumor mutational burden (TMB) were 74.75 and 41, and 2.47 and 1.3 mut/Mb, respectively (Figure 1B, Table 1). Missense mutations represented the most abundant variant classification (Figure 1C). The average microsatellite instability (MSI) was 0.47% in the 12 lung cancer patients (Table 2). The top 10 significantly mutated genes were identified, with *MUC4*, *EGFR*, *TP53*, *MUC16*, *OR1F1*, *MAGEL2*, *LRRC4C*, *AGXT*, *ADGRV1*, and *ABCC4* having a false discovery rate Q-value (FDR) < 0.1 and mutation rates of 50%, 42%, 25%, 25%, 17%, 17%, 17%, 17%, 17%, and 17%, respectively (Figure 1D).

Next, the top 10 significantly mutated genes in the tumor tissues from the lung cancer patients were identified, including *EGFR*, *MUC4*, *MUC18*, *TP53*, *ABCC4*, *ADGRV1*, *AGXT*, *LRRC4C*, *MAGEL2*, and *OR1F1*, with FDR < 0.1 and mutation rates of 50%, 42%, 25%, 25%, 17%, 17%, 17%, 17%, 17%, and 17%, respectively (Figure 2A). Screening mutations in the TCGA database of lung cancer revealed the top 10 mutated genes as *TP53*, *CSMD3*, *LRP1B*, *EGFR*, *SYNE1*, *ASPM*, *MUC16*, *TTN*, *ZFHX4*, and *DNAH5*, with mutation rates of 61%, 48%, 39%, 35%, 35%, 26%, 26%, 26%, 26%, and 22% (Figure 2B). The tumor tissues from both the cohorts in Zhoushan Archipelago and the

Table 1. The details of mutation status in 12 lung cancer tissues.

Tumor sample	Frame-shift deletions	Frame-shift insertions	In-frame deletions	Missense mutations	Nonsense mutations	Nonstop mutations	Total	TMB (mu/Mb)
S1	0	0	0	30	3	1	34	1.2
S2	0	0	0	26	2	0	28	1.0
S3	12	1	0	227	19	0	259	8.6
S4	0	0	2	44	2	0	48	1.6
S5	3	1	1	38	0	1	44	1.4
S6	2	1	0	109	9	0	121	4
S7	4	3	4	22	3	0	36	1.2
S8	3	2	5	26	2	0	38	1.2
S9	4	4	4	119	2	0	133	4.4
S10	3	8	4	80	3	0	98	3.2
S11	1	3	3	18	1	0	26	0.8
S12	6	2	3	20	1	0	32	1.0

TMB: Tumor Mutational Burden.

Table 2. The microsatellite instability status in 12 lung cancer tissues.

Sample	Total number of sites	Number of somatic sites	Score (%)
S1	21,416	1	0
S2	9,533	0	0
S3	17,402	59	0.34
S4	19,921	5	0.03
S5	20,487	6	0.03
S6	19,942	1	0.01
S7	19,867	38	0.19
S8	20,367	116	0.57
S9	18,696	389	2.08
S10	20,487	174	0.85
S11	20,070	132	0.66
S12	21,232	181	0.85

TCGA database harbored 193 genes including *TP53*, *EGFR*, and *MUC16* mutations.

Clinical characteristics such as age, gender, tobacco smoking history, tumor location, tumor size, lymph node metastasis, histological subtype, and pathological stage showed no significant association with the mutation status of the tumor tissues from the lung cancer patients (p all > 0.05).

Mutation status in pleural effusion samples from lung cancer patients

The targeted genomic DNA sequencing of pleural effusion samples (aligned to the hg19 reference genome) yielded the following average quality metrics: a mapping rate of 99.88%, an on-target rate of 70.0%, and a duplication rate of 53.02%. Across the targeted exonic regions, the average sequencing depth reached 3,000×, with a mean coverage depth of 3,589.8×. Furthermore, 92.05% of the targeted bases achieved a minimum coverage depth of 10×. In the pleural effusion cohort, a total of 259 mutations were identified in 136 genes,

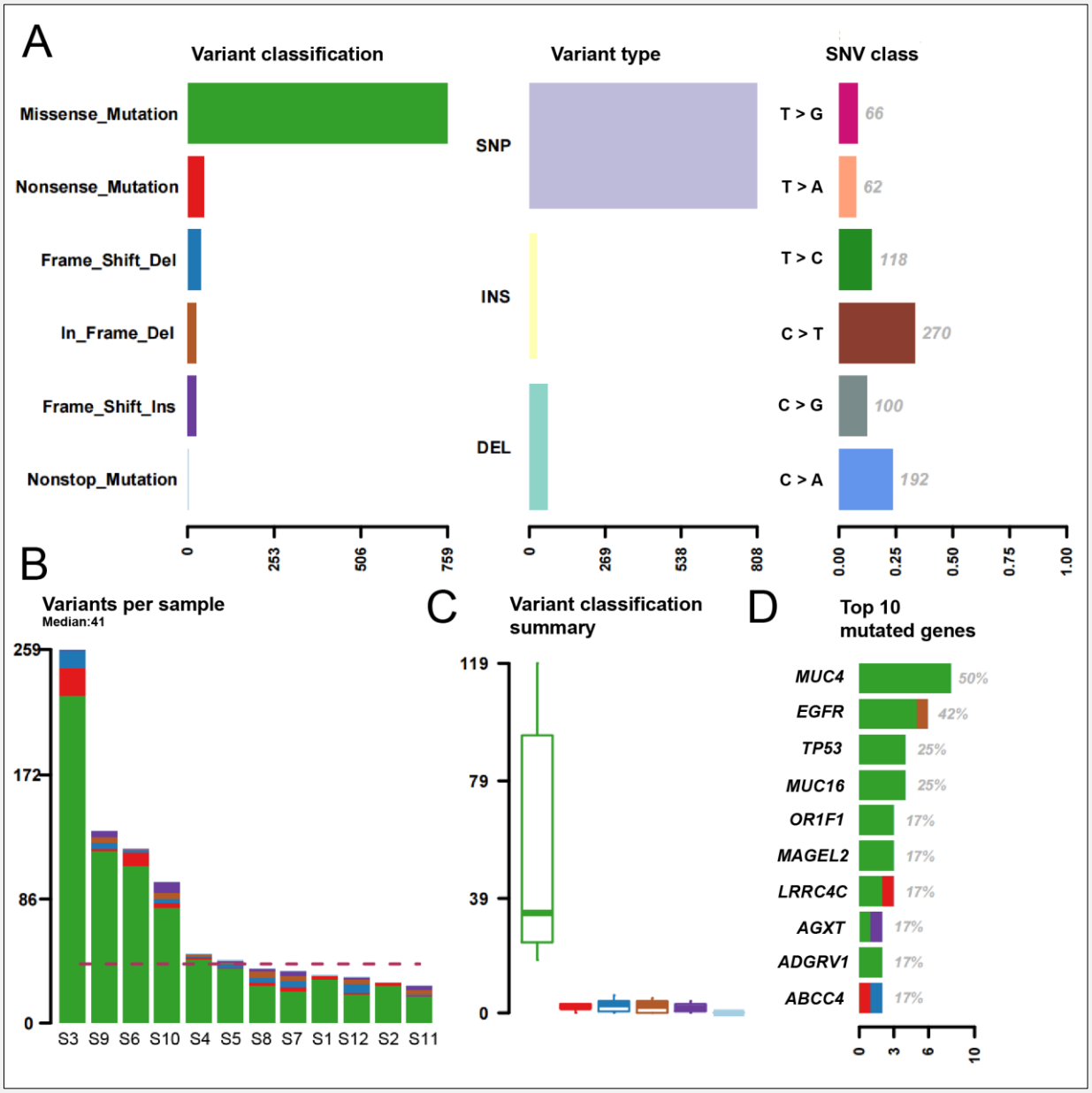

Figure 1. The gene mutation status in the tumor tissues from 12 lung cancer patients.

A: Variant classification, variant type, and single nucleotide variation (SNV) class, B: Variants per sample, C: variant classification summary, D: the top 10 significantly mutated genes.

comprising 88 frame-shift deletions, 15 frame-shift insertions, 13 in-frame deletions, 1 in-frame insertion, 123 missense mutations, 13 nonsense mutations, and 6 splice-site mutations (Figure 3A). According to the mutation classification, SNPs accounted for 54.05% (140/259), insertions accounted for 6.18% (16/259), deletions accounted for 39.00% (101/259), and double nucleotide polymorphisms accounted for 0.47% (2/259, Figure 3A). The dominant SNV class was C > T, accounting for 43.51% (114/262), followed by T > C (14.50%, 38/262) and C > G (14.12%, 37/262; Figure 3A). The details of the mutation statuses in each sample are presented in Supplementary Table 3. The mean and median mutation counts were 35.75 and 21.5 (Figure 3B, Sup-

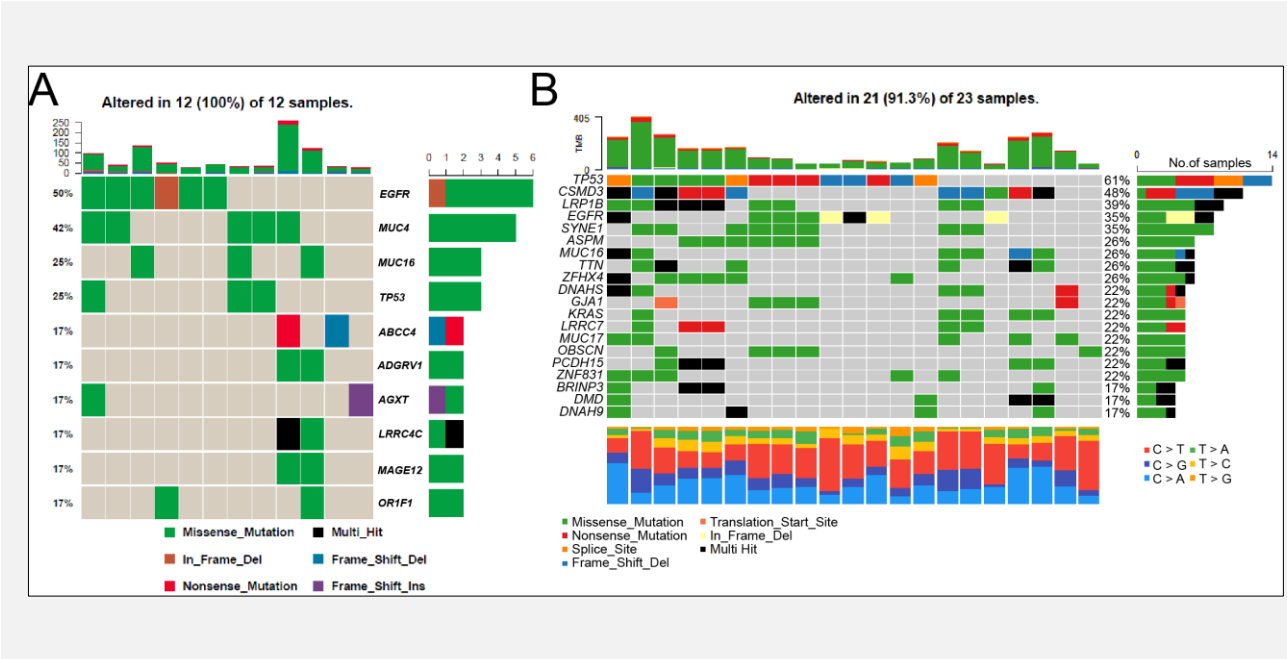

Figure 2. The significantly mutated genes in tumors tissues.

A: The top 10 significantly mutated genes in the tumor tissues from 12 lung cancer patients. B: The top 20 significantly mutated genes in the 23 lung cancer patients included in the Cancer Genome Atlas database.

plementary Table 3), with missense mutations being the most abundant variant classification (Figure 3C). The average MSI was 0.38% in the 12 lung cancer patients (Supplementary Table 4). The top 10 significantly mutated genes were identified, including *SETD8*, *GNAQ*, *CIC*, *TERT*, *STAT5A*, *SLX4*, *PAX5*, *RARA*, *ATM*, and *ARID1B*, with FDR < 0.1 and mutation rates of 75%, 42%, 42%, 50%, 50%, 50%, 50%, 33%, 42%, and 33%, respectively (Figure 3D).

Additionally, the top 10 significantly mutated genes in the pleural effusion samples from the lung cancer patients were *SETD8*, *KMT2C*, *ANKRD11*, *ARID5B*, *PIK-3CA*, *FCGR3A*, *RYR2*, *MED12*, *RNF43*, and *H3F3A*, with FDR < 0.1 and mutation rates of 62%, 36%, 25%, 25%, 17%, 17%, 17%, 17%, 17%, and 17%, respectively (Figure 4).

Mutation difference between tumor tissues and pleural effusion samples

We subsequently compared the mutation status between the tumor tissues and pleural effusion samples. The results revealed that the frequency of missense mutations in the tumor tissues was significantly greater than that in the pleural effusion samples ($p = 0.0002$, Figure 5A). In contrast, the rate of frame-shift deletions in the tumor tissues was markedly less than that in the pleural effusion samples ($p < 0.0001$, Figure 5B). Other variant types showed comparable frequencies between the tumor tissues and the pleural effusion samples. The MSI score of the tumor tissue samples was greater than that of the pleural effusion samples ($p = 0.0015$, Figure 5C). Furthermore, the mutational rates of oncogenic genes varied between the tumor tissues and the pleural effusion samples. Among all tissue-detected mutations, 11 common mutations (*EGFR*, *TP53*, *APOB*, *COL3A1*, *CXCR4*, *DNMT3A*, *DSG2*, *EWSR1*, *KMT2D*, *NRG1*, and *TSC2*) overlapped with those in the pleural effusion samples. The pleural effusion samples harbored 125 individual mutations not identified in the tumor tissues (Figure 5D), suggesting that the sequencing information obtained from the pleural effusion samples could partly reveal the status of the tumor tissues and address tumor heterogeneity to complement the tumor tissues.

DISCUSSION

The application of next-generation sequencing technology for tumor tissue sequencing has proven effective in guiding targeted precision therapy and improving therapeutic outcomes [11]. However, a considerable number of lung cancer patients are diagnosed at advanced stages, often presenting with pleural effusion. In recent years, liquid biopsy techniques have emerged as valuable tools for assessing individual tumor responses in lung cancer patients [12-15]. Pleural effusion, in particular, has been recognized as a valuable liquid biopsy analyte, providing insights into genomic alterations in

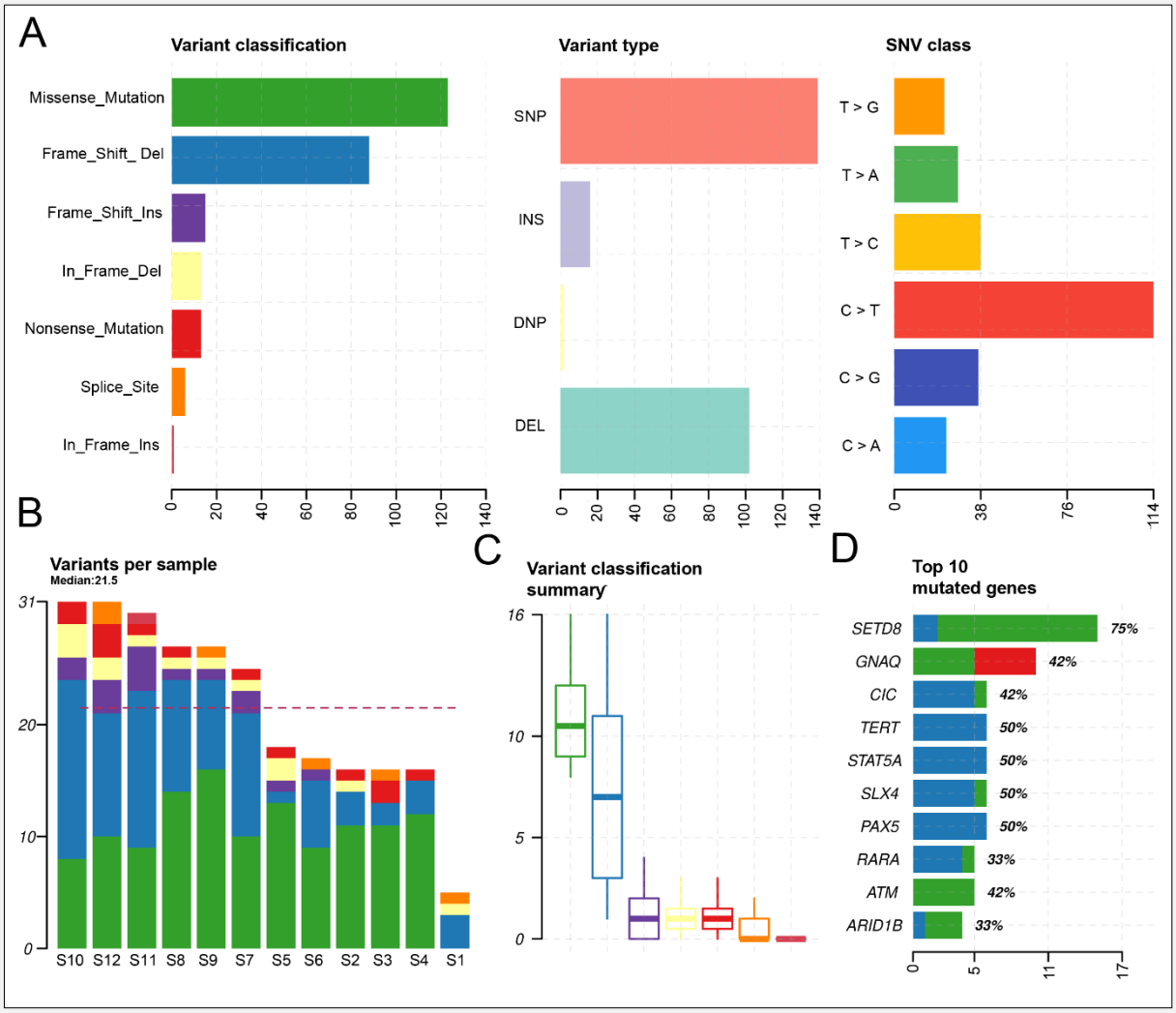

Figure 3. The gene mutation status in the pleural effusion samples from 12 advanced lung cancer patients.

A: Variant classification, variant type, and single nucleotide variation (SNV) class, B: variants per sample, C: variant classification summary, D: the top 10 significantly mutated genes.

clinical settings [16,17].

In our study conducted in the Zhoushan Archipelago in eastern China, exon mutation profiling of tumor tissues from 12 lung cancer patients and pleural effusion samples from 12 advanced lung cancer patients was performed. Our findings revealed a notable difference in the mutation patterns between tumor tissues and pleural effusion samples. Specifically, we observed a higher frequency of missense mutations in the tumor tissue samples compared to the pleural effusion samples. Conversely, the rate of frame-shift deletions in the tumor tissues was significantly less than that in the pleural effusion samples. Notably, among all tissue-detected mutations, 11 common mutations (*EGFR*, *TP53*, *APOB*, *COL3A1*, *CXCR4*, *DNMT3A*, *DSG2*, *EWSR1*, *KMT2D*, *NRG1*, and *TSC2*) were identified, overlapping with those found in the pleural effusion samples. These findings suggest that pleural effusion samples can provide additional insights into tumor heterogeneity and complement the information obtained from tumor tissues.

In our screening of tumor tissues from Zhoushan Archipelago patients, a comprehensive analysis identified a total of 897 mutations, with SNPs accounting for 89.86%. The prevalent SNV class observed was C > T. The mean mutation counts and TMB were 74.75 and 2.47 mut/Mb, respectively. Additionally, the average

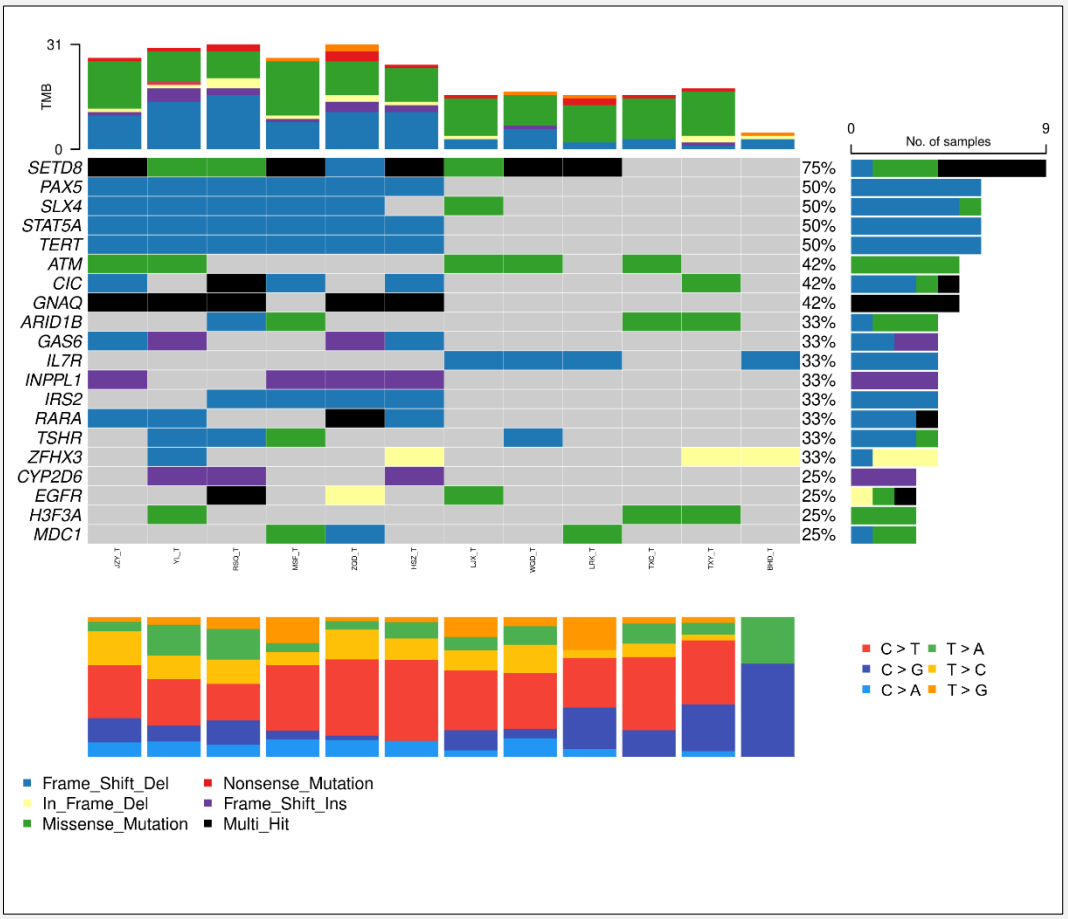

Figure 4. The top 20 significantly mutated genes in the pleural effusion samples from 12 advanced lung cancer patients.

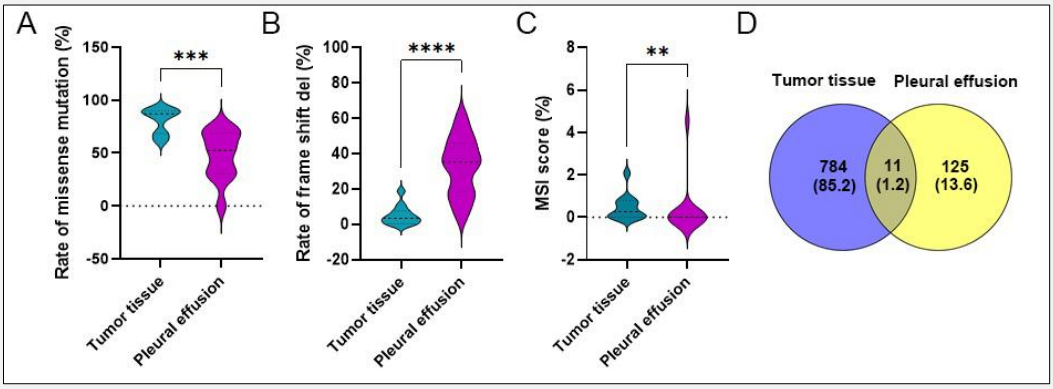

Figure 5. Difference of mutation status between the tumor tissues and pleural effusion samples from lung cancer patients.

A: Rate of missense mutations, B: frame shift deletions and C: microsatellite instability (MSI) scores between tumor tissues and pleural effusion samples from lung cancer patients, D: the overlap of mutated genes in tumor tissues and pleural effusion samples.
** p < 0.01, *** p < 0.001, **** p < 0.0001.

MSI was determined to be 0.47%. The top 10 significantly mutated genes in the lung cancer patients from this cohort included *EGFR*, *MUC4*, *MUC18*, *TP53*, *ABCC4*, *ADGRV1*, *AGXT*, *LRRC4C*, *MAGEL2*, and *OR1F1*. Comparison with the TCGA database revealed that both the Zhoushan Archipelago cohort and the TCGA cohort shared 193 genes, including mutations in *TP53*, *EGFR*, and *MUC16*. This result suggests analogous molecular events in lung cancer across different populations.

However, it is noteworthy that some frequently mutated genes identified in other studies were not observed in our Zhoushan Archipelago cohort. A study of lung adenocarcinoma patients from eastern Asia has highlighted *EGFR*, *TP53*, and *KRAS* as the most frequent driver genes [18]. Another Chinese patient study has reported *TP53*, *EGFR*, and *KRAS* as the most common mutated genes [19]. An additional investigation has identified high mutation frequencies in *TP53*, *EGFR*, *CREBBP*, *KMT2C*, *MUC2*, *DNMT3A*, *LRP1B*, *MUC4*, and *CDC27* [20, 21], partly similar to our results of the cohort from Zhoushan Archipelago.

Notably, our study identified novel gene mutations in lung cancer patients, including mutations in *ADGRV1*, *AGXT*, and *OR1F1*. The adhesion G protein-coupled receptor V1 (*ADGRV1*) encodes a protein that features a seven-transmembrane receptor domain, exhibits calcium-binding capability, and is predominantly expressed in the central nervous system. Mutations in this gene have been associated with Usher syndrome type 2 and familial febrile seizures [22]. Mutations in *ADGRV1* have been reported in gastric cancer and hepatocellular carcinoma, and correlated with immune infiltration in ovarian cancer [23-25]. A recent study further indicated that *ADGRV1* mutation is associated with worse progression-free survival (PFS) in stage I-III micropapillary lung adenocarcinoma [26]. The enzyme alanine-glyoxylate aminotransferase (AGT) encoded by the *AGXT,* catalyzes the conversion of glyoxylate to glycine in hepatic peroxisomes [27,28]. *AGXT* mutations, which involve in amino acid metabolism, have been identified in MSI-high gastric cancer and/or colorectal cancer [29]. Meanwhile, olfactory receptor family 1 subfamily F member 1 (*OR1F1)* has been found to be down-regulated in breast cancer, acting as a suppressor in metastasis progression [30]. In contrast, high OR1F1 protein expression was detected as an independent adverse prognostic factor in esophageal squamous cell carcinoma, suggesting that its role may be context-dependent and warrants further investigation [31].

Our exploration of somatic mutations in the pleural effusion cohort revealed distinct patterns between the supernatant DNA and cell pellets, with mutations detected in 89.1% of the supernatant DNA samples and only 54.3% of the cell pellets reported in two studies [32,33]. Focusing on the supernatant, we identified 259 mutations, with SNPs accounting for 54.05% and the primary SNV class being C > T. The mean and median mutation counts were 35.75 and 21.5, respectively, and the average MSI was 0.38%. The top 10 significantly mutated genes in the lung cancer patients with pleural effusion included *SETD8*, *KMT2C*, *ANKRD11*, *ARID-5B*, *PIK3CA*, *FCGR3A*, *RYR2*, *MED12*, *RNF43*, and *H3F3A.* This cohort's genomic landscape demonstrated unique alterations compared to studies in other populations. Previous research has shown that pleural effusion samples commonly harbor mutations in genes like *EGFR*, *KRAS*, *BRAF*, and *MET* along with additional alterations in genes such as *SMARCA4*, *PIK3CA*, *ERBB2*, *KMT2A*, *ALK*, and *NF1* [34]. Furthermore, our findings align with studies highlighting driver mutations in supernatant DNA, including *EGFR*, *KRAS*, *BRAF*, and *MET* [32]. Notably, one study has identified the presence of *EML4-ALK*, *ROS1*, *HER2*, and *RET* in lung adenocarcinoma patients with pleural effusion, showcasing a diverse genomic landscape in this cohort [35]. In addition, a Taiwanese study has identified 18 cancer driver genes in lung cancer patients with pleural effusion, partially overlapping with our findings [4]. Importantly, our study identified *ANKRD11* as a potential novel somatic mutation in lung cancer patients with pleural effusion. Moreover, *ANKRD11*, known as a tumor suppressor in breast cancer, has demonstrated co-activator roles with p53 [36,37].

The sensitivity of detecting actionable mutations in pleural effusion from advanced lung cancer patients has been reported to surpass that of plasma, emphasizing its potential clinical relevance [38,39]. Tong and colleagues have further highlighted the similarity in the tumor mutational burden between the supernatant of pleural effusion and matched tumor tissue [39]. In our current study, we observed a distinct mutational distribution between tumor tissue and pleural effusion samples. Specifically, missense mutations were more prevalent in tumor tissue, whereas frame shift deletions occurred at a significantly higher frequency in pleural effusion. This divergence likely reflects differences in mutational processes and selective pressures across the two microenvironments. In local tumor tissue, missense mutations may confer a selective growth advantage and undergo clonal expansion for oncogenic "driver" events that often result in gain-of-function or context-dependent alterations. In contrast, pleural effusion samples accumulated more frame shift deletions, which may be tolerated as passenger mutations under relaxed selective constraints. Alternatively, frame shift-mediated inactivation of tumor suppressors or differentiation-related genes could provide an adaptive advantage within the effusion niche.

Furthermore, comparing unmatched tumor tissues and pleural effusion samples, only 11 common mutations were identified, suggesting a lower rate of overlap compared to other studies [32,34]. This finding underscores the importance of future research focusing on matched samples to enhance the reliability of genomic data.

The current study has some inherent limitations that must be addressed. First, obtaining matched tumor tissues and pleural effusion samples was challenging due

to the advanced stage at which the lung cancer patients with pleural effusion are typically diagnosed, limiting the opportunity for surgery. Second, the small population size in Zhoushan Archipelago further constrained sample acquisition, and the sample size was insufficient for robust analysis, thus introducing potential bias into the study. Third, the tumor mutational burden was not calculated, and several common driver mutations were not detected in this cohort, reflecting a partial representation of the genomic profile in lung cancer patients from Zhoushan Archipelago.

CONCLUSION

In conclusion, we demonstrated the exon mutations in tumor tissues from 12 lung cancer patients and in pleural effusion samples from 12 other advanced lung cancer patients. It was observed that the number of missense mutations in the tumor tissues was significantly greater than that in the pleural effusion samples, while the rate of frame-shift deletions in the tumor tissues was markedly less than that in the pleural effusion samples. Among all detected mutations, 11 common mutations (*EGFR*, *TP53*, *APOB*, *COL3A1*, *CXCR4*, *DNMT3A*, *DSG2*, *EWSR1*, *KMT2D*, *NRG1*, and *TSC2*) overlapped with those in the pleural effusion samples. This study suggests that pleural effusion could serve as an alternative liquid biopsy for mutation detection, complementing data from tumor tissues and guiding individualized targeted therapy for patients with advanced lung cancer.

Availability Data and Materials:
The dataset used or analyzed in the current study is available from the corresponding author upon reasonable request.

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Declaration of Generative AI in Scientific Writing:
The author(s) declare that no Generative AI was used in the creation of this manuscript.

Declaration of Interest:
The authors have declared that there are no conflicts of interest.

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