Original Article

Correlation analysis between driver gene mutation and clinicopathological features in lung adenocarcinoma based on real-world cumulative clinical data

Sheng Lu[1#], Aotian Guo[1#], Haichuan Hu[1], Xinxin Ying[1], Yao Li[1], Zhengwei Huang[1], Wangjue Xu[2], Shen Tao[1], Xiaotong Hu[3], Na Yan[4], Xuan Zhang[4], Dan Shen[4], Takaaki Sasaki[5], Surein Arulananda[6], Ken Onodera[7], Zhengfu He[1]

[1]Department of Thoracic Surgery, Sir Run Run Shaw Hospital, School of Medicine, Zhejiang University, Hangzhou, China; [2]Department of Thoracic Surgery, Longyou People's Hospital, Longyou, China; [3]Department of Pathology, Sir Run Run Shaw Hospital, School of Medicine, Zhejiang University, Hangzhou, China; [4]Key Laboratory of Digital Technology in Medical Diagnostics of Zhejiang Province, Dian Diagnostics Group Co., Ltd., Hangzhou, China; [5]Department of Internal Medicine, Division of Respiratory Medicine and Neurology, Asahikawa Medical University, Asahikawa, Hokkaido, Japan; [6]Department of Medical Oncology, Monash Health, Clayton, VIC, Australia; [7]Department of Thoracic Surgery, Institute of Development, Aging and Cancer, Tohoku University, Sendai, Miyagi, Japan

Contributions: (I) Conception and design: Z He, S Lu; (II) Administrative support: Z He; (III) Provision of study materials or patients: Z He, S Tao; (IV) Collection and assembly of data: S Lu, A Guo, H Hu, Z Huang, X Hu; (V) Data analysis and interpretation: S Lu, N Yan, X Zhang, D Shen; (VI) Manuscript writing: All authors; (VII) Final approval of manuscript: All authors.

[#]These authors contributed equally to this work.

Correspondence to: Zhengfu He, MD. Department of Thoracic Surgery, Sir Run Run Shaw Hospital, School of Medicine, Zhejiang University, 3 East Qingchun Road, Hangzhou 310016, China. Email: hezhengfu@zju.edu.cn.

Background: Driver genes are essential predictors of targeted therapeutic efficacy. Detecting driver gene mutations in lung adenocarcinoma (LUAD) patients can help to screen for targeted drugs and improve patient survival benefits. This study aims to investigate the mutation characterization of driver genes and their correlation with clinicopathological features in LUAD.

Methods: A total of 440 LUAD patients were selected from Sir Run Run Shaw Hospital between July 2019 and September 2022. Postoperative tissue specimens were analyzed for gene mutations using next-generation sequencing technology, focusing, including epidermal growth factor receptor *EGFR*, *ALK*, *ROS1*, *RET*, *KRAS*, *MET*, *BRAF*, *HER2*, *PIK3CA* and *NRAS*. At the same time, clinicopathological data were collected and organized for multidimensional correlation analysis.

Results: Of 440 LUAD patients, driver gene mutations were not detected in 48 patients. The proportion of patients with driver gene mutations was as high as 89.09%. The top three driver genetic mutations were *EGFR*, *KRAS*, and *MET*. Sixty-nine types of *EGFR* mutations were detected and distributed in the protein tyrosine kinase catalytic domain (56, 81.16%), Furin-like cysteine-rich region (9, 13.04%), receptor binding domain (3, 4.35%), and *EGFR* transmembrane domain (1, 1.45%). Single gene locus mutation occurred in 343 LUAD patients, but the mutation gene types covered all tested genes. Our findings showed that *EGFR* mutations were more commonly observed in non-smoking and female patients (P<0.01), *KRAS* mutations were more prevalent in male patients and smokers (P<0.01), ROS1 mutations had larger tumor diameters (P<0.01) and *RET* mutations were more prevalent in smokers (P<0.05).

Conclusions: LUAD patients exhibit diverse genetic mutations, which may co-occur simultaneously. Integrated analysis of multiple mutations is essential for accurate diagnosis and effective treatment of the disease. The use of NGS can significantly expand our understanding of gene mutations and facilitate integrated analysis of multiple gene mutations, providing critical evidence for targeted treatment methods.

Keywords: Clinicopathological features; next-generation sequencing (NGS); gene mutation; target therapy; lung adenocarcinoma (LUAD)

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Introduction

Approximately 80% of lung cancer cases are classified as non-small cell lung cancer (NSCLC). The most common subtype of NSCLC is lung adenocarcinoma (LUAD), which accounts for approximately 55% of NSCLC (1). LUAD has a higher incidence and case fatality rate compared to other types of malignant tumors and exhibits a high level of genetic heterogeneity (2,3). Despite significant improvements in clinical treatment through traditional surgery and the combination of radiotherapy and chemotherapy, overall efficacy and prognosis remain limited. In the past 20 years, with the development of tumor molecular biology and the gradual deepening of gene research, the diagnosis and treatment of LUAD have undergone significant changes (4-7). The development and application of targeted drugs have extended survival and improved LUAD patients' quality of life (8). The carcinogenic mutation of the epidermal growth factor receptor (*EGFR*) tyrosine kinase domain showed a significant clinical response to the first-generation *EGFR* tyrosine kinase inhibitor (TKI) gefitinib in 2004 (9), ushering in the era of targeted treatment for NSCLC. Three years later, the discovery of *ALK* rearrangement broadened the scope of targeted genomic changes in the disease (10). Subsequently, some other driver events with strong transformation potential were reported, including somatic oncogenic mutations of *ROS1*, *RET*, *BRAF*, gene insertion of *HER2*, and exon 14 skipping of *MET* proto-oncogene (8,11). Driver genes are essential predictors of targeted therapeutic efficacy. Detecting driver gene mutations in LUAD patients can help to screen for targeted drugs and improve patient survival benefits (12-15). This study analyzed the mutations of driver genes (*EGFR*, *KRAS*, *ALK*, *ROS1*, *RET*, *MET*, *BRAF*, *HER2*, *PIK3CA* and *NRAS*) in LUAD and their correlation with clinical pathological features, providing a theoretical basis for promoting multi-gene joint detection. We present this article in accordance with the MDAR reporting checklist (available at https://tlcr.amegroups.com/article/view/10.21037/tlcr-24-409/rc).

Highlight box

Key findings

- The study identified driver gene mutations in a substantial 89.09% of the 440 lung adenocarcinoma (LUAD) patients.
- Epidermal growth factor receptor (*EGFR*) mutations exhibited varied distribution, with the majority in the protein tyrosine kinase catalytic domain. Single gene locus mutations occurred in 343 patients, covering all tested genes.
- *EGFR* mutations were more prevalent in non-smoking and female patients, while *KRAS* mutations were associated with male patients and smokers. *ROS1* mutations correlated with larger tumor diameters, and *RET* mutations were more common in smokers.

What is known and what is new?

- Prior knowledge includes the prevalence of *EGFR*, *KRAS*, and other driving mutations in LUAD. The association of *EGFR* mutations with non-smoking and female patients, and *KRAS* mutations with smokers, aligns with existing understanding.
- Identifying a broad spectrum of mutations can help clinicians tailor treatments to individual patients, particularly for those with rare or co-occurring mutations. The association of *ROS1* mutations with larger tumor diameters and *RET* mutations with smoking represents novel insights into the clinicopathological features associated with specific mutations.

What is the implication, and what should change now?

- Understanding the diverse genetic mutations in LUAD patients is crucial for personalized treatment strategies. The association of mutations with clinical features suggests potential biomarkers for prognosis and treatment response.
- Next-generation sequencing may be beneficial for clinicians to identify a broader spectrum of mutations for LUAD patients.

Methods

Patients and tumor samples

In this retrospective study, a total of 440 LUAD patients who met the inclusion criteria were selected from Sir Run Run Shaw Hospital. The inclusion criteria were: (I) the result of cytological or pathological diagnosis was LUAD; (II) patient was not receiving any radiotherapy/chemotherapy treatment before the operation; (III) traceability of the multi-gene joint test result could be implemented. Patients were excluded based on the following criteria: (I) patients without clinical pathological information; (II) gene testing experiment failed. All surgical lung tissue specimens were classified by two independent pathologists based on histological results. The study was conducted in accordance with the Declaration of Helsinki

(as revised in 2013), and was approved by the Ethics Committee of Sir Run Run Shaw Hospital (No. 20230488). Informed consent has been taken from the participants before taking part.

Sample preparation and DNA extraction

LUAD tissue samples, with a tumor content exceeding 20%, were collected during surgical procedures. The QIAamp DNA FFPE Tissue Kit (Qiagen, Dusseldorf, Germany) was used to extract these samples, adhering to the manufacturer's guidelines. DNA concentration was evaluated using Qubit 3.0 (Thermo Fisher Scientific, Waltham, USA), while DNA size distribution was analyzed with the Qsep100 system (Bioptic, Taiwan, China). These standardized protocols were used to ensure accurate and reproducible results.

Next-generation sequencing (NGS) library preparation

This study utilized the CleanPlex™ sequencing kit (Paragon Genomics, Silicon Valley, USA) following the optimized protocol provided by the manufacturer to generate sequencing libraries. The current study employed multiplex PCR to target 10 LUAD-associated genes and enrich 200 g of genomic DNA. Beckman Coulter's Agencourt AMPure XP beads were used to purify the DNA library. The NGS library was further purified and quantified using the Qubit 1X dsDNA Assay Kit from Thermo Fisher Scientific. Fragment size distribution was analyzed using Bioptic's Qsep100 in Taiwan, China. Genetic testing was conducted in a laboratory certified by the College of American Pathologists (CAP) to ensure that high quality standards were met. A panel of 10 LUAD-associated genes was employed to identify single nucleotide variants, deletions, insertions, delins mutations, duplications, and fusion.

Sequencing and bioinformatics analysis

Library sequencing was performed on the Illumina NextSeq 500 platform (Illumina, San Diego, USA), achieving an average depth of over 1,000×. Variants with a frequency of at least 1% were designated as mutations. Paired-end sequencing data from libraries in FASTQ format were aligned to the human genome (hg19) using Burrows-Wheeler Aligner (BWA-MEM). To identify potential fusion detection points, the coverage depth of adjacent sites and fusion breakpoints was calculated. ANNOVAR21, along with the hg19 reference genome, standard databases, and functional prediction programs, were used to perform SNV and indel annotation.

Statistical analysis

All analyses in this academic paper were conducted via R version 4.1.1 (2021-08-10). Continuous variables were presented using mean and standard deviation or median and interquartile ranges, and comparisons were made using the Student's *t*-test or the Mann-Whitney *U* test. Subgroup analyses were conducted using the Chi-square test, with Fisher's exact test applied for small sample sizes. Kendall's tau coefficient was employed for correlation analyses. All analyses conducted in this paper were two-tailed, and a significance level of $P \leq 0.05$ was used as the standard criterion for determining statistical significance. This paper addresses an audience of our peers and esteemed instructors.

Results

Clinicopathological characteristics of 440 LUAD patients

Four hundred and forty LUAD patients were enrolled in this study. In this study, there was no gender bias at the onset of LUAD (available online: https://cdn.amegroups.cn/static/public/tlcr-24-409-1.xlsx). There were 213 (48.41%) male patients and 227 (51.59%) female patients with a male-to-female ratio of 1:1.0657. The age range of the LUAD patients was 26 to 89 years, with a median age of 65 years. More than 60% of all patients with LUAD were elderly aged >60 years old. Three-quarters of LUAD occurred in patients without a history of smoking. A minority of LUAD patients showed multiple pulmonary lesions, while the majority (over 80%) exhibited singular lesions. Sixty percent of the patients were diagnosed with stage I LUAD. The main patient groups of LUAD were tumor diameter ≤3 cm or without any lymph node metastasis or any distant metastasis. The incidence of the right lung was 1.2 times higher than that of the left lung. The upper lobe incidence rate was 1.5 times higher than that of the lower lobe (*Table 1*).

Driver gene mutation characteristics

Among the 440 LUAD patients, 48 did not have detectable driver gene mutations (available online: https://cdn.amegroups.cn/static/public/tlcr-24-409-1.xlsx). The

Table 1 Demographic and clinical characteristics of 440 patients with lung adenocarcinoma

Characteristics	No. of patients	%
Gender		
Male	213	48.41
Female	227	51.59
Age, years		
≤60	165	37.50
>60	275	62.50
Smoke		
Yes	36	8.18
No	333	75.68
Quit	56	12.73
Unknown	15	3.41
Lesions		
Single	366	83.18
Multiple	74	16.82
Diameter		
≤3	358	81.36
>3 and ≤5	62	14.09
>5	20	4.55
Stage		
Stage I	264	60.00
Stage II	19	4.32
Stage III	56	12.73
Stage IV	41	9.32
Unknown	60	13.64
Distant metastasis		
None	349	79.32
Yes	91	20.68
Lymph node metastasis		
Yes	85	19.32
No	355	80.68
Location of lesions		
Left	196	44.55
Right	237	53.86
Left & right	5	1.14
Others	2	0.45
Location of lesions (pulmonary lobe)		
Upper lobe	223	50.68
Lower lobe	146	33.18
Upper & lower lobe	38	8.64
Others	33	7.50

percentage of patients with a driver gene mutation was 89.09%. 392 LUAD patients carried at least one driver gene mutation, including 343 patients with a single gene mutation and 49 patients with two or more driver gene mutations. The results of the multi-gene analysis show that *EGFR* (299, 67.95%) had the highest mutation rate among 10 genes, followed by *KRAS* (33, 7.50%), *MET* (26, 5.91%), *HER2* (24, 5.45%), *ALK* (17, 3.86%), *RET* (14, 3.18%), *ROS1* (10, 2.27%), *BRAF* (10, 2.27%), *PIK3CA* (8, 1.82%) and *NRAS* (1, 0.23%) (*Figure 1*).

Among the 299 patients with *EGFR* mutations, 260 patients carried only one *EGFR* mutation site, 38 patients carried two *EGFR* mutation sites, and only one patient carried three *EGFR* mutation sites simultaneously. The most common *EGFR* mutations were L858R and exon19 deletion, which accounted for 46.82% and 39.13% of patients with *EGFR* mutations respectively. The *EGFR* T790M mutation was found in six TKI-naive LUAD patients. Three rare *EGFR* mutations, G719X (7, 2.34%), S768I (6, 2.01%), and L861X (6, 2.01%) were identified. We detected 69 different *EGFR* mutation sites in the 299 patients with *EGFR* mutations which were distributed in the protein tyrosine kinase catalytic domain (56, 81.16%), Furin-like cysteine-rich region (9, 13.04%), receptor binding domain (3, 4.35%), and *EGFR* transmembrane domain (1, 1.45%) (*Figure 2*). Forty-three patients with *EGFR* mutations also carried other driver mutations (*Table 2*). The gene with the highest incidence of EGFR co-mutation was MET [14], while other genes were *ERBB2* [7], *RET* [6], *PIK3CA* [5], *ROS1* [5], *ALK* [3] and *KRAS* [1].

We also observed 23 patients with fusion mutations. *ALK* fusion occurred in 15 patients, with a total of 5 companion genes, the most common of which was *EML4* in 12 cases, and other rare companion genes were *DCTN1*, *AC011239.1*, *STRN* and *FAM179A* (Table S1). There were three subtypes of *EML4-ALK* fusion, including three cases of *E6:A20*, five cases of *E13:A20* and one case of *E18:A20*. RET fusion occurred in four patients, and the partner genes were *KIF13A*, *KIF5B*, *KCND2* and *LDLR*. *ROS1* fusion mutation was detected in four patients, with one patient fusing with two partner genes, *STK39* and *EZR*, and other partner genes being *FAM65B*, *PKHD1* and *GLI3*.

Associations between driver gene mutation and clinicopathological features in LUAD

In this study, driver gene mutations were more likely to occur in female LUAD patients (P=0.03). *EGFR* mutations

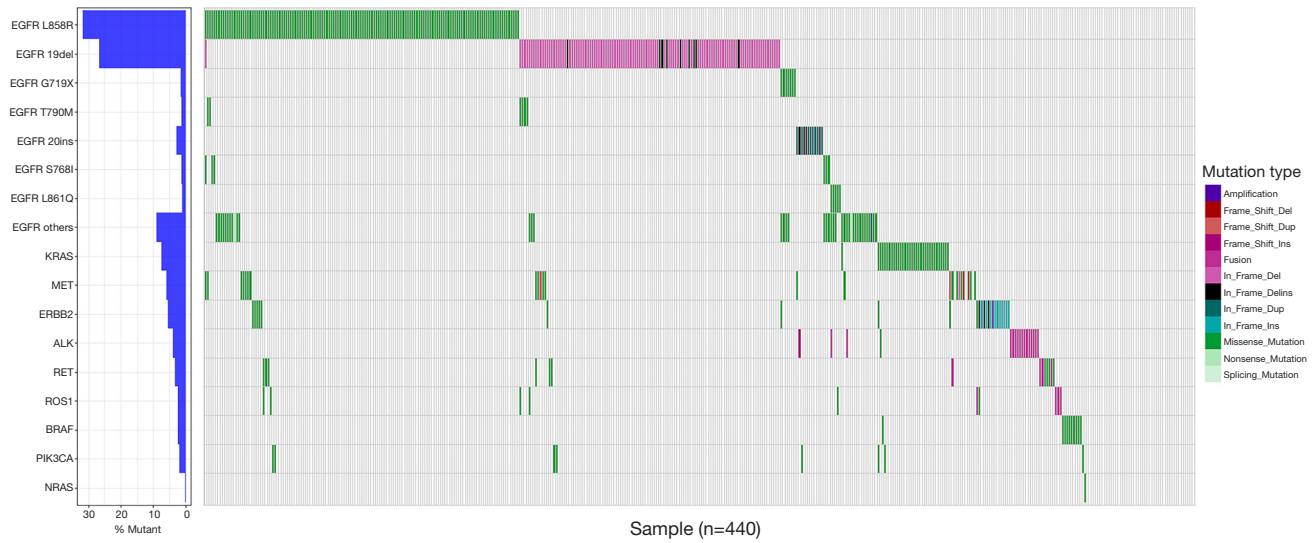

Figure 1 Genetic mutation in 440 LUAD patients. LUAD, lung adenocarcinoma; *EGFR*, epidermal growth factor receptor.

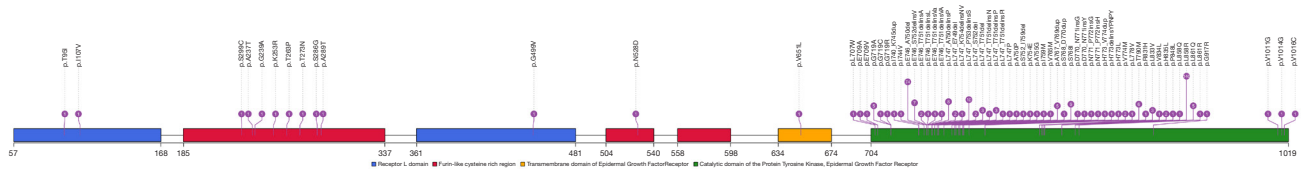

Figure 2 Distribution of mutations in EGFR functional domains. EGFR, epidermal growth factor receptor.

Table 2 *EGFR* mutations co-occurred with other genetic alterations

Co-mutated gene	No. of patients	% of *EGFR* mutation (n=299)	% of LUAD (n=440)
EGFR + MET	14	4.68	3.18
EGFR + ERBB2	7	2.34	1.59
EGFR + RET	6	2.01	1.36
EGFR + PIK3CA	5	1.67	1.14
EGFR + ROS1	5	1.67	1.14
EGFR + ALK	3	1.00	0.68
EGFR + KRAS	1	0.33	0.23
EGFR + ROS1 + RET	1	0.33	0.23
EGFR + MET + RET	1	0.33	0.23

EGFR, epidermal growth factor receptor; LUAD, lung adenocarcinoma.

also tended to occur in female patients (P=0.0036). There were differences in driving gene mutations among LUAD patients with different smoking histories (P=0.057), but they were not significant. However, EGFR mutations were prevalent in LUAD patients without a history of smoking (P=0.0005). No significant differences were detected in driver gene or *EGFR* mutations concerning age, lesion placement, lesion quantity, tumor size, presence of lymph node metastasis, lesion metastasis, and clinical stage (*Table 3*). The Kendall correlation significance test indicated that EGFR mutations were mutually exclusive with mutations in *KRAS*, *ALK*, *ERBB2*, and *BRAF* (P<0.001) (refer to *Figure 3*). *EGFR* mutations were predominantly observed in women and non-smokers (P<0.01), whereas *KRAS* mutations were more prevalent among male smokers (P<0.01).

Table 3 Comparison of driving gene mutations among different clinical indicator groups

Characteristics	Gene mutation			EGFR mutation		
	Negative	Positive	P value	No	Yes	P value
Gender			0.03			0.0036
Male	31	182		83	130	
Female	17	210		58	169	
Age, years			0.43			0.33
≤60	21	144		58	107	
>60	27	248		83	192	
Smoking history			0.057			0.0005
No	30	303		93	240	
Yes	4	32		16	20	
Quit	11	45		29	27	
Location of lesions (pulmonary lobe)			0.44			0.31
Upper lobe	27	196		67	156	
Lower lobe	12	134		55	91	
Upper & lower lobe	5	33		12	26	
Location of lesions			0.81			0.93
Left	21	175		63	133	
Right	26	211		76	161	
Left & right	1	4		2	3	
No. of primary focus			0.87			0.84
Single lesion	39	326		116	249	
Multiple lesions	9	65		25	49	
Tumor diameter, cm			0.12			0.056
≤3	37	321		108	250	
>3 and ≤5	6	56		22	40	
>5	5	15		11	9	
Lymphatic metastasis			0.49			0.95
Yes	7	78		27	58	
No	41	314		114	241	
Tumor metastasis			0.59			0.50
Yes	8	83		26	65	
No	40	309		115	234	
Tumor stage			0.42			0.65
Stage I	31	233		87	177	
Stage II	0	19		4	15	
Stage III	5	51		20	36	
Stage IV	5	36		12	29	

EGFR, epidermal growth factor receptor.

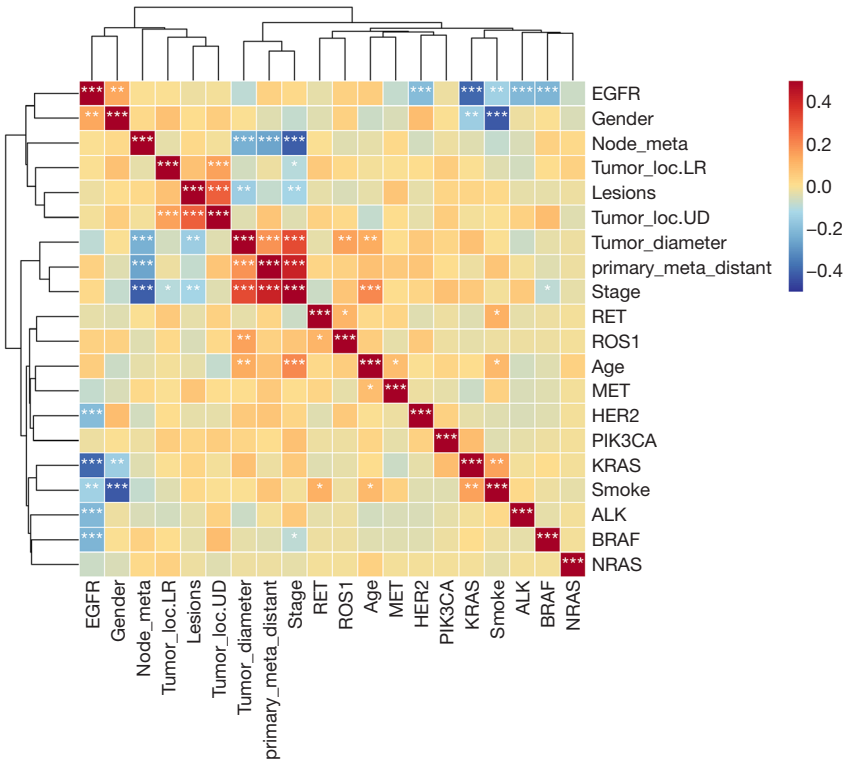

Figure 3 Kendall correlation analysis between gene mutations and clinical features. Test of significance of the Kendall correlation coefficient: *, P<0.05; **, P<0.01; ***, P<0.001. *EGFR*, epidermal growth factor receptor.

ROS1 mutations and *RET* mutations were prone to occur simultaneously (P<0.05). Patients with *ROS1* mutations had larger tumor diameters (P<0.01). *RET* mutations were more prevalent in individuals who smoke (P<0.05).

Discussion

Mutations in driver genes play a crucial role in determining the response to targeted therapies in LUAD patients. Targeted therapy guided by gene mutations significantly improves treatment choices and survival benefits for LUAD patients (11). In addition to commonly activating *EGFR* mutations, targets such as *ALK* rearrangement, *ROS1* rearrangement, *RET* rearrangement, *BRAF V600E*, *MET* exon 14 skipping mutation, and *KRAS G12C* have gradually been approved for targeted therapy (8). The number of gene mutations associated with targeted therapy has increased from one gene to several genes (16,17). Therefore, a comprehensive examination of genetic mutations in patients is essential. Multi-gene targeted sequencing is a rapid and cost-effective detection method that can provide comprehensive recommendations for LUAD-targeted drugs. In this analysis, we employed a multi-gene targeted sequencing technology to comprehensively evaluate driver gene mutations in 440 LUAD patients. The top three driver genetic mutations were *EGFR*, *KRAS*, and *MET*. Typically, *EGFR* and *KRAS* mutations do not occur together, but we observed a patient with concurrent EGFR and *KRAS* mutations, suggesting primary resistance to *EGFR* inhibitors. In addition, we identified five patients with *EGFR L858R* mutations or exon 19 deletions accompanied by *EGFR* T790M mutations, indicating resistance to the first and second-generation *EGFR*-TKIs. These findings suggest that clinicians need to carefully select *EGFR*-TKIs targeted therapy, and consider the patient's benefits when developing treatment plans. Furthermore, we found that 27 patients detected only one *KRAS* hotspot mutation, G12X or Q61H. Nine patients carried *BRAF* mutations, six patients had *MET* mutations, 12 patients developed *HER2* insertion mutations, and 17 patients formed gene fusions that included 12 *ALK* fusions, three *RET* fusions, and two *ROS1* fusions. It is important to note that these patients carried only one gene mutation. There was a rare case of *ALK* fusion, *DCTN1/ALK* (D24: A20). Gao *et al.* (18)

reported one case of *DCTN1/ALK* fusion that achieved partial response after receiving cabozantinib therapy. Adding to the complexity of genomic landscapes in LUAD, we report a patient with a novel fusion involving STK39 and EZR, where EZR's coiled-coil domain contrasts with the absence of such a structure in STK39. This discrepancy underscores the need for further exploration of STK39's role in this fusion context and highlights the importance of investigating these gene fusions as potential driver mutations. It is necessary to detect multiple gene mutations in LUAD patients to increase the chances of obtaining potentially beneficial information about target genes and to reduce the likelihood of negative results from single-gene testing. Previous studies have shown that LUAD's molecular characteristics are influenced by factors such as the environment, family and lifestyle (19,20). Some studies emphasized the close relationship between environmental factors, such as gender and smoking, and LUAD molecular characteristics (21-23). Wu *et al.* (24) analyzed 506 NSCLC patients, and the results showed that the *EGFR* mutation rate in non-smoking patients was higher than that in smoking patients and mutation rate was higher in female patients than male patients. An analysis by An *et al.* (25) on 524 NSCLC patients revealed that the gene most frequently altered in non-smoking adenocarcinoma patients was *EGFR*. Our analysis revealed a notable correlation between gene mutations and clinicopathological factors including gender, smoking history, and lesion site. Specifically, *EGFR* mutations were significantly more frequent among non-smokers and female patients ($P<0.01$). However, our analysis indicated that *KRAS* mutations were more frequent in male patients and smokers ($P<0.01$). The observation that *ROS1* mutations are associated with larger tumor diameters may be due to the aggressive nature of *ROS1*-driven tumors, which activate pathways that promote cell proliferation and survival, leading to increased tumor growth. Studies have shown that *ROS1* rearrangements can activate downstream signaling pathways, such as PI3K/AKT and MAPK/ERK, which are crucial for cell proliferation and survival (26,27). It is plausible that similar mechanisms could be involved in tumors with *ROS1* mutations, although further research is needed to elucidate this association. Interestingly, while our study found a higher prevalence of *RET* mutations in smokers, previous literature indicates that *RET* fusions occur more frequently in non-smokers (28). This discrepancy suggests different underlying mechanisms for *RET* mutations and fusions. Smoking-related carcinogens are known to increase the overall mutational burden, potentially leading to *RET* mutations through DNA-damaging effects. However, *RET* fusions may not be as strongly influenced by smoking status. Future studies are necessary to clarify these associations and to understand the specific effects of smoking on *RET* mutations and fusions.

Targeted therapies have significantly advanced the treatment of LUAD by focusing on specific genetic alterations (7,8). *EGFR* mutations are effectively targeted by drugs such as gefitinib, erlotinib, and osimertinib. *ALK* rearrangements respond well to inhibitors like crizotinib, alectinib, and brigatinib. *ROS1* rearrangements can be treated with crizotinib and entrectinib. For *BRAF V600E* mutations, the combination of dabrafenib and trametinib is effective. Additionally, *MET* amplifications and METex14 skipping mutations can be targeted by drugs like capmatinib and tepotinib. These therapies offer significant improvements in patient outcomes and quality of life.

Meanwhile, identifying a broad spectrum of mutations can help clinicians tailor treatments to individual patients, particularly for those with rare or co-occurring mutations, as seen in *Table 2*. In LUAD, *EGFR* mutations may co-occur with other genetic alterations, impacting disease progression, treatment response, and prognosis. Notably as reports, *TP53* mutations are commonly found alongside *EGFR* mutations, often indicating a poorer prognosis and potentially influencing the response to *EGFR* inhibitors (29). *PIK3CA* mutations can co-exist with *EGFR* mutations, possibly affecting the efficacy of *EGFR*-targeted therapies and contributing to disease progression (30). Although rare, *ALK* rearrangements can also occur alongside *EGFR* mutations, typically resulting in reduced efficacy of single-agent targeted therapies and necessitating combined treatment approaches. While *KRAS* mutations are generally considered mutually exclusive with *EGFR* mutations, their occasional co-occurrence indicates complex tumor biology and typically poorer prognosis. *MET* amplification is a known mechanism of acquired resistance to EGFR inhibitors, requiring alternative therapies such as *MET* inhibitors (31). HER2, BRAF mutations and *ROS1* rearrangements, though less common, also represent significant challenges, often indicating resistance to standard *EGFR*-targeted treatments. There are also reported cases of triple alterations involving *EGFR*, *ROS1*, *RET*, *KRAS* and *ALK* mutations, further complicating treatment strategies and necessitating highly personalized therapeutic approaches (32-34). Understanding these co-occurring genetic alterations is crucial for developing personalized treatment strategies and improving clinical outcomes for

LUAD patients.

This study has several limitations. Firstly, the targeted sequencing panels used had limited capacity, potentially overlooking certain mutations in LUAD patients. We have recognized the importance of *NTRK* gene fusions and their potential impact on patient management. *NTRK* gene fusions are recognized as significant oncogenic drivers in various cancers, including LUAD. These fusions involve the *NTRK1*, *NTRK2*, and *NTRK3* genes, leading to the constitutive activation of the TRK signaling pathway, which promotes tumorigenesis (35). The inclusion of *NTRK* gene fusions in comprehensive genomic profiling is therefore essential for identifying patients who may benefit from TRK inhibitor therapies. Future studies will include *NTRK* gene fusions to provide a more complete landscape of actionable genetic alterations in LUAD, ensuring that all potential therapeutic targets are considered. Whole exome sequencing may be a recommended method for a comprehensive understanding of gene alterations. While our study provides valuable insights into the molecular mutation characteristics of LUAD patients and their correlation with clinicopathological features, our study lacked follow-up information on the patients, thus preventing us from analyzing the relationship between molecular mutation characteristics and prognosis. Further research is needed to elucidate the relationship between co-occurring genetic mutations and their impact on LUAD prognosis and treatment outcomes. Collective insights from these investigations will contribute to the development of more effective targeted therapy strategies for LUAD patients. Understanding the prognostic implications of these mutations is crucial for guiding treatment decisions and predicting patient outcomes. Additionally, the widespread implementation of CT lung cancer screening in China has led to the early detection and treatment of an increasing number of early-stage lung cancer cases. This screening practice contributes significantly to the observed predominance of early-stage LUAD in our study. Similarly, the higher age range for *EGFR* mutations and the predominance of stage I cancers in our cohort reflect this selection bias. Most NSCLC cases present at a later stage (stage III or IV) and are not suitable for surgical resection. For advanced-stage NSCLC patients, we typically perform pathological biopsy to seek targeted therapy for optimal benefit. our study was limited to a single-center retrospective analysis, which may introduce selection bias and limit the generalizability of our findings to broader populations.

Conclusions

In conclusion, our study found that LUAD patients in large centre in China exhibited diverse genetic mutations, which may co-occur simultaneously. Integrated analysis of multiple mutations is essential for accurate diagnosis and effective treatment of this disease. The use of NGS can significantly expand our understanding of gene mutations and facilitate integrated analysis of multiple gene mutations, providing critical evidence for targeted treatment methods. Despite the limitations of this study, these findings hold promise for improving clinical decision-making for LUAD management in patients. Further research is needed to elucidate the relationship between co-occurring genetic mutations and their impact on LUAD prognosis and treatment outcomes. Collective insights from these investigations will contribute to the development of more effective targeted therapy strategies for LUAD patients.

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Footnote

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Data Sharing Statement: Available at https://tlcr.amegroups.com/article/view/10.21037/tlcr-24-409/dss

Peer Review File: Available at https://tlcr.amegroups.com/article/view/10.21037/tlcr-24-409/prf

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