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Novel causes and assessments of intrapulmonary metastasis

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ABSTRACT

Background: This study utilized next-generation sequencing (NGS) to analyze genetic information and gene mutation-related loci in ground glass nodules (GGN) with multiple (≥2) lesions. Pathological findings were then correlated to distinguish between multiple primary lung cancers (MPLC) and pulmonary metastasis (PM).
Methods: A cohort of 20 individuals who underwent surgical resection for ground glass nodules (GGN) was included. Final diagnosis and restaging were determined based on the analysis of clinical characteristics and NGS single-target genetic detection.
Results: Histopathological, immunohistochemical staining, and NGS analyses identified 48 tissue samples from 20 cases of multiple malignant nodules. A total of 66 gene mutations were identified, with four cases classified as PM. Notably, four patients with intrapulmonary metastases exhibited concurrent mutations in the epidermal growth factor receptor (EGFR) (50 %) and Kirsten ratsarcoma viral oncogene homolog (KRAS). Comparatively, the prevalence of EGFR mutations in PM patients was significantly higher than that in primary lesions.
Conclusion: Genomic analysis of multiple lung adenocarcinomas enables the determination of the clonal status of tumor cells across various lesions. When gene mutation sites in multiple lesions are identical and mutation abundance is significantly elevated, early intrapulmonary metastasis may be diagnosed.

1. Introduction

In recent years, the widespread adoption of early-stage lung cancer screening has resulted in an increased number of non-small cell lung cancer (NSCLC) patients being diagnosed early through low-dose CT (LDCT) scans. While surgical treatment remains the preferred option, it is not uncommon for the pathological tissues obtained after surgery from multiple lesions to be malignant. The genetic differences between these multiple malignant lesions significantly impact the diagnosis, staging, postoperative adjuvant treatment, and prognosis of the disease. The term "multiple primary lung cancers" (MPLC) is used to describe the simultaneous or successive occurrence of two or more primary malignancies in different parts of the lung within the same patient [1]. Differentiating between MPLC and pulmonary metastasis (PM) is crucial for predicting prognosis and determining appropriate treatment strategies in affected patients [2]. By consulting resources like the OMIM database, we confirmed that gene is strongly linked to several genetic diseases, particularly lung cancer and inherited metabolic disorders.

When multiple tumors exhibit histological similarity, distinguishing between MPLC and PM becomes a challenging task. This distinction holds clinical significance as it improves prognosis assessment and treatment accuracy, considering the diverse treatment strategies and prognoses associated with these conditions. Patients meeting surgical criteria undergo genetic testing of excised lung tissue to identify gene mutation sites and match appropriate targeted drugs. Tissue-based genetic testing is still considered the gold standard [3]. Next-generation sequencing (NGS) provides high-throughput detection of multiple gene statuses and mutations in vivo, covering genes such as EGFR, TP53, KRAS, MET, PTEN, and others, along with information on mutation sites. This paper explores the feasibility of distinguishing MPLC and PM from a molecular biology perspective, utilizing NGS sequencing and pathological diagnosis of pulmonary nodules. Based on this, a re-evaluation of lung cancer staging is conducted, and relevant anti-tumor therapy is discussed.

2. Methods

2.1. Study design and patients

A total of 20 patients who underwent multifocal resection of lung cancer from June 2022 to December 2022 were collected from the Department of Thoracic Surgery, General Hospital of Northern Theater

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2.2. Inclusion criteria

(i) Age between 18 and 70 years old; (ii) Preoperative imaging data indicating a high likelihood of malignant tumors with the presence of two or more lesions; (iii) Adequate cardiopulmonary function for surgery; (iv) Absence of a family history of tumors, and preoperative examinations showing no evidence of distant metastasis, including head CT, abdominal ultrasound, enhanced CT of the chest and abdomen, and bone ECT or whole-body pet-CT; (v) Intraoperative frozen pathology revealing carcinogenesis or focal carcinogenesis, and postoperative pathological results confirming adenocarcinoma; (vi) NGS testing conducted on surgically resected specimens only after the patient and their family had provided informed consent for genetic testing post-surgery.

2.3. Exclusion criteria

(i) Individuals experiencing secondary recurrence or metastasis from other systemic tumors; (ii) Those with pathological findings indicative of carcinoma in situ; (iii) Individuals who had undergone preoperative chemoradiotherapy.

2.4. Outcome measures

Next-generation sequencing (NGS) was employed to identify mutation sites in key driver genes (EGFR, TP53, KRAS, MEK) and tumor suppressor genes. The study recorded information on the location of mutated gene lesions, the number and type of lesions, maximum tumor diameter, histological type, pleural invasion, and the mutation status of genes related to single-target analysis.

2.5. Test criteria

Yishan Biological Laboratory provided the NGS detection results, employing NexSeq550, CanPatrol rare blood cell separator, and Cytostainer 48 automatic stainer as the detection instruments. The kits utilized in the process were registered and CE marked by CFDA, enabling the detection of a comprehensive range of gene mutations. This included EGFR gene amplification, precise EGFR mutation typing (18, 19, 20, 21), ALK rearrangement, KRAS mutations (in exon 2 (codon 12/13) and exon 3 (codon 61) of KRAS gene), HER-2 gene amplification, and PIK3CA mutation (refer to Table 1).

2.6. Bioinformatics analysis methods

1 Data Generation and Preprocessing

Table 1
Common gene mutations in lung cancer.

ABL1	AKT1	ALK	APC[a]	ATM	BRAF[a]	CDH1
CDKN2A	CSF1R	CTNNB1	EGFR[a]	ERBB2	ERBB4	EZH2
FBXW7	FGFR1	FGFR2	FGFR3	FLT3	GNA11	GNAQ
GNAS	HER2[a]	HRAS	IDH1	IDH2	JAK2	JAK3
KDR	KIT	KRAS[a]	MEK[a]	MLH1	MPL	NOTCH1
NPM1	NRAS	PDGFRA	PIK3CA[a]	PTEN	PTPN11	RB1
RET	SMAD4	SMARCB1	SMO	SRC	STK11	TP53[a]
VHL						

[a] During this study, a notable observation was the frequent mutation of specific genes in lung adenocarcinoma. These encompassed mutations occurring in both tumor suppressor genes and oncogenes, indicating a recurring pattern of mutations in these cancer-related genes.

2.6.1. Sequencing platform and quality control

Whole exome sequencing (WES) or targeted sequencing (Panel) was performed using the Illumina NovaSeq 6000. Raw data quality was assessed with FastQC, and low-quality reads ($Q < 20$) or adapter contamination were filtered using Trimmomatic.

2.6.2. Sequence alignment and deduplication

Reads were aligned to the hg38 reference genome using BWA-MEM, and PCR duplicates were removed with GATK MarkDuplicates.

2 Variant Detection and Annotation

2.6.3. Somatic mutation calling

SNV/Indel: GATK Mutect2 (tumor-normal paired samples) detected mutations with filtering thresholds of VAF $\geq$5 % and sequencing depth $\geq 50\times$.

CNV/SV: Copy number variations (CNVs) were analyzed by FACETS, and structural variants (SVs) were identified using Manta.

2.6.4. Functional annotation and database filtering

Variants were functionally annotated (e.g., missense mutations, splice sites) with ANNOVAR. Clinically relevant mutations were prioritized using COSMIC v96 and ClinVar, while polymorphisms (gnomAD frequency >1 %) were excluded.

2.6.5. Somatic variant screening criteria

1 Technical Filtering

Coverage Depth: Retained loci required $\geq 50\times$ depth in tumor samples and $\geq 30\times$ in normal samples.

Read Support: Mutations required $\geq$5 unique supporting reads to mitigate amplification bias.

Cross-Contamination Control: VerifyBamID excluded samples with inter-sample contamination (threshold <3 %).

2 Biological Filtering

Driver Gene Prioritization: Known oncogenic mutations in lung cancer-related genes (EGFR, KRAS, TP53) were retained based on OncoKB.

2.7. Functional impact grading

High-confidence variants: Non-synonymous mutations, frameshift Indels, or splice site ($\pm$2 bp) variants.

Excluded variants: Synonymous mutations and noncoding region variants (unless in known regulatory regions).

2.8. Analysis process for differentiating MPLC and PM

Step 1 **Molecular Profile Comparison**

Driver Mutation Concordance: PM was favored if lesions shared $\geq$1 identical driver mutation; MPLC was supported by non-overlapping driver mutation profiles.

Mutational Signature Analysis: SigProfiler extracted mutational signatures (e.g., APOBEC hypermutation, smoking-related Signature 4). Discordant signatures suggested multiple primaries.

Step 2 Clonal Phylogenetic Reconstruction

Subclonal Structure Analysis: PyClone-VI inferred clonal populations. Shared ancestral clones indicated PM, while independent clonal structures supported MPLC.

Phylogenetic Divergence: PhyloWGS-generated trees with inter-

lesion branch distances >80 % genetic divergence suggested independent origins.

Step 3 Multimodal Data Integration

Histopathological Validation: Lesion subtypes (e.g., adenocarcinoma vs. squamous carcinoma) and PD-L1 expression differences were compared.

Imaging Correlation: CT/PET-CT assessed nodule distribution (PM: peripheral/random; MPLC: central).

Step 4 Statistical Validation

Clonal Independence Testing: A Bayesian model (Lee et al., 2022) calculated independent occurrence probabilities (significance: $p < 0.05$).

2.9. Critical quality control

Tumor Purity Correction: ABSOLUTE or ICHORCNA estimated tumor purity; only samples with ≥20 % purity were analyzed.

Batch Effect Control: Horizon Discovery reference standards were included per batch to ensure technical consistency.

2.10. Statistical analysis

Quantitative data were expressed as mean ± standard deviation (x ± s) if they followed a normal distribution after a normality test. The comparison between groups was conducted using the *t*-test. For continuous data with a skewed distribution, they were expressed as median and upper and lower quartiles [M (P25, P75)], and the comparison between groups utilized the rank sum test. Categorical data were presented as frequencies and percentages, and the chi-square test (χ^2) was employed for group comparisons. Fisher's exact test was used when the theoretical frequency of cells met specific criteria. Statistical significance was set at $P < 0.05$. All statistical analyses were performed using R software version 4.2.2(Comprehensive R Archive Network, Alcatel-Lucent, NZ).

Driver Mutation Concordance Test: Fisher's exact test was used to assess the overlap probability of driver mutations between lesions, with a significance threshold of $p < 0.05$. Clonal Independence Validation: A Bayesian framework (Lee et al., 2022) calculated the posterior probability of independent clonal origins, and permutation testing (1000 iterations) with Benjamini-Hochberg correction addressed multiple hypothesis testing. Mutational Signature Discordance: Cosine similarity quantified inter-lesion signature similarity (threshold: <0.8).

Justification of Sample Size.

The sample size was determined via G*Power 3.1 based on pre-experimental effect size (Cohen's $d = 0.6$, $\alpha = 0.05$, $\beta = 0.2$), ensuring ≥80 % statistical power.

Statistical Limitations.

(i) Retrospective design introduces selection bias, requiring prospective validation; (ii) Tumor heterogeneity may affect clonal evolution inference; (iii) Targeted sequencing panels may miss low-frequency driver variants.

3. Results

3.1. Clinical and pathological characteristics of cases

Between June 2022 and December 2022, a cohort of 20 patients received an initial diagnosis of multiple malignant nodules following simultaneous surgical resection. The mean age of the patients was (58.9 ± 6.1) years, comprising 7 males and 13 females. Postoperative pathological and immunohistochemical staining results confirmed the presence of adenocarcinoma (ADC) and minimally invasive adenocarcinoma (MIA), with comprehensive clinical data encompassing preoperative examinations, imaging data, and postoperative treatments.

A total of 48 pulmonary nodule resection specimens were collected from the 20 patients, revealing that 15/20 patients had 2 nodules, 3 patients had 3 nodules, 1 patient had 4 nodules, and 1 patient had 5/7 nodules diagnosed as malignant. NGS testing samples were submitted upon the patients' signing of informed consent for genetic testing, meeting the submission conditions: 1. Tumor cell content ≥20 %; 2. DNA concentration ≥5 ng/μl (refer to Fig. 1).

This picture presents the results obtained through Next-generation Sequencing (NGS) analysis, focusing on genes associated with single-target analysis. The analysis included critical genes such as EGFR, TP53, KRAS, MEK, and others. The results offer valuable insights into the mutation status of these specific genes, providing a comprehensive view of the genetic landscape associated with single-target abnormalities.

Among the 20 cases, females constituted the majority (65.0 %), with 40.0 % of patients having a history of smoking and undergoing surgical resection. Nine cases were diagnosed with multiple minimally invasive adenocarcinomas (MIAs), three cases with a combination of adenocarcinoma (ADC) and MIA, and all eight cases with ADC alone. Among the ADC and MIA patients, three had bilateral multiple nodules, while the remaining 17 had unilateral multiple nodules.

60 % of tumors were in different lobes on the same side, and malignant tumors were infrequent in both lobes (15 %). Some tumors were found in the same lobe on the same side (25 %). Approximately 10 % of patients had more than two tumors, with one patient undergoing bilateral wedge resection and subtotal lingual resection of the left upper lobe, resulting in seven lung tissues containing lesions. This procedure confirmed malignancy (adenocarcinoma) in five cases. The mean tumor size was 1.2 (0.9, 1.8) cm. Statistical analysis revealed no significant differences in gender, age, smoking history, and comorbidities among the patients ($p > 0.05$) (refer to Table 2).

3.2. Genetic alterations

Mutations in the epidermal growth factor receptor (EGFR) gene were identified in 13 out of the 20 enrolled patients (65 %), observed in 24 specimens. Among adenocarcinoma (ADC) lesions, 7 exhibited exon 21L858R, and 5 lesions exhibited deletion missense mutations in exon 19. Copy number amplification was identified in only one ADC sample. Notably, NGS single target associated gene results suggested EGFR gene alterations in nine minimally invasive adenocarcinoma (MIA) lesions, with some specimens exhibiting EGFR with TP53 mutation.

Anaplastic lymphoma kinase (ALK) fusions were not found in any specimen, while Kirsten rat sarcoma (KRAS) mutations were present in 5 lesions (10.4 % of 48), and MEK/MEK1 mutations in 5 cases, with concurrent detection in one patient. (refer to Table 3).

3.3. Programmed cell death-ligand 1 (PD-L1) expression

No gene mutations were detected in seven lesions, and no positive mutations were found in normal lung tissue in any of the 20 patients. Statistical analysis revealed significant differences in mutation abundance and histological type between pulmonary metastasis (PM) and multiple primary lung cancer (MPLC) groups. However, no statistical differences were observed in tumor location, lesion type, tumor diameter, and cell infiltration. In cases where the same mutation site was identified, particularly with EGFR mutations, there was a statistically significant difference in mutation abundance values in metastatic lesions ($p < 0.05$). Subsequent opinions from at least two pathologists in our hospital's Department of Pathology suggested a higher likelihood of metastatic lesions between the two lesions (refer to Table 3).

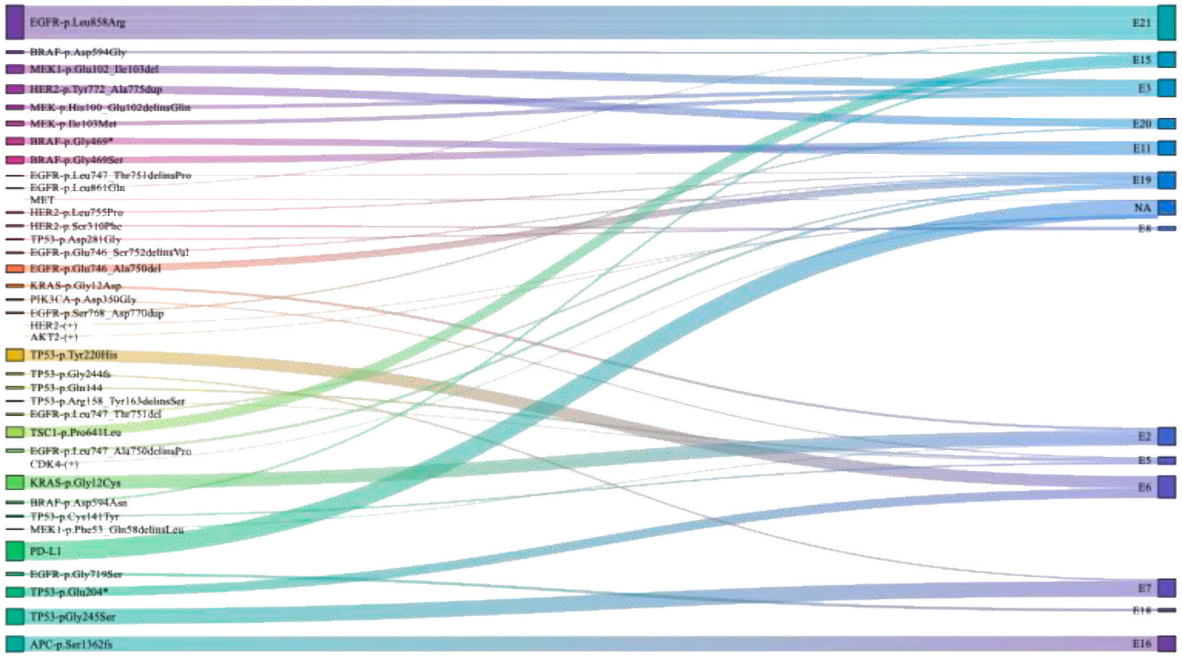

Fig. 1. Ngs results (genes related to single targets).

Table 2
Summary of baseline patient characteristics.

Parameter	Total *(n = 20)*	MPLC *(n = 16)*	PM *(n = 4)*	*P value*
Gender				0.63
Female	13 (65)	10 (62.5)	3 (75)	
Male	7 (35)	6 (37.5)	1 (25)	
Age(yr)	58.9 ± 6.1	58.8 ± 6.3	59 ± 6.3	0.66
Smoking				0.61
Never	12 (60)	9 (56.2)	3 (75)	
Ever	8 (40)	7 (43.8)	1 (25)	
Complications				0.77
Never	5 (25)	4 (25)	1 (25)	
Ever	15 (75)	12 (75)	3 (75)	
LVEF	0.63 ± 0.02	0.63 ± 0.02	0.63 ± 0.01	0.91
Pulmonary function				
FEV1	2.4 ± 0.6	2.4 ± 0.7	2.6 ± 0.4	0.29
MVV	99.8 ± 22.7	98.4 ± 24.3	105.2 ± 15.7	0.51

Data are number (percentage) or (Mean ± Standard Deviation).
LVEF, Left Ventricular Ejection Fractions.
FEV1, Forced Expiratory Volume in the first second.
MVV, Maximal Voluntary Ventilation.

Table 3
Clinicopathological characteristics.

variable	total (n = 66)	PM (n = 4)	MPLC (n = 62)	*P value*
Mutation abundance	9.8 (6.6, 14.9)	29.6 (23, 32)	9.4 (5.3, 12.2)	0.03
Tumor location				0.68
RUL	15 (31.2)	1 (25)	14 (31.8)	
RML	5 (10.4)	0 (0)	5 (11.4)	
RLL	10 (20.8)	0 (0)	10 (22.7)	
LUL	11 (22.9)	2 (50)	9 (20.5)	
LLL	7 (14.6)	1 (25)	6 (13.6)	
Type of lesion				0.77
SN	3 (6.2)	0 (0)	3 (6.8)	
mGGO	26 (54.2)	2 (50)	24 (54.5)	
pGGO	19 (39.6)	2 (50)	17 (38.6)	
Histological type				0.05
MIA	24 (50)	1 (25)	23 (52.3)	
ADC	23 (47.9)	2 (50)	21 (47.7)	
PM	1 (2.1)	1 (25)	0 (0)	
Maximum tumor diameter	1.2 (0.9, 1.8)	1.8 (1.5, 2)	1.2 (0.9, 1.7)	0.29
Cell infiltration				0.34
PM	25 (52.1)	1 (25)	24 (54.5)	
MPLC	23 (47.9)	3 (75)	20 (45.5)	

RUL, Right Upper lobe; RML, Right Middle lobe; RLL, Right lower lobe; LUL, Left Upper lobe; LLL, Left lower lobe; SN, Solid nodules; mGGO, Mixed ground-glass nodules; pGGO, Pure ground-glass nodules; ADC, adenocarcinoma; MIA, minimally invasive adenocarcinoma; MPLCs, multiple primary lung cancers; PM, pulmonary metastasis.

4. Discussion

The onset and progression of lung cancer represent a complex biological phenomenon involving multiple genes and stages. Tumor heterogeneity, often considered a complicating factor, constraint, or potential limitation in molecular tumor investigations, warrants attention. Temporal heterogeneity, denoting variations in mutations during disease progression and metastasis, has been extensively studied in lung cancer [4–10]. Concurrent or subsequent tumors, sharing rare genetic bases or branching drivers, likely stem from a common ancestor. However, common mutations, especially backbone drivers, may also arise independently due to chance [14,19,20]. The concordance of EGFR and KRAS mutations, especially when validated through sensitive methods like NGS testing in larger cohorts, is noteworthy [4,8].

In Asia, multiple primary lung cancers (MPLCs) are more prevalent in non-small cell lung cancer (NSCLC). Adenocarcinoma predominates, with approximately 50 % of Chinese NSCLC patients showing a positive rate of epidermal growth factor receptor (EGFR) gene mutations. Among these, the classical mutations EGFR L858R (20 %) and EGFR 19del (20 %) are most common [11–13]. Our mutation analysis results indicate that EGFR has the highest mutation rate (50 %), followed by TP53 (approximately 14.6 %), KRAS (approximately 10.4 %), MEK/MEK1 (10.4 %), and HER2 (8.3 %). Temporal heterogeneity, assessed by comparing mutations in primary tumors and paired metastatic lesions, reveals a high concordance rate in driver genes, supported by the earlier detection of primary tumors in the patient's medical history. Our findings align with research on KRAS, EGFR, BRAF, and TP53 mutation consistency in primary and metastatic lung cancer [14].

The current diagnosis of MPLC relies on the eighth edition of the

American Joint Committee on Cancer (AJCC) staging manual [15] and the TNM staging in the latest Chinese Guidelines for the Diagnosis and Treatment of Integrated Oncology (CACA). Accurate differentiation between MPLC and pulmonary metastasis (PM) is crucial, as they significantly differ in pathological stage, prognosis, and post-surgery treatment outcomes. Nicole Ezer [16] highlights the utility of next-generation sequencing (NGS) in cases with consistent low-frequency mutations, such as TP53, for assessing tumor clonality. However, in cases of consistent mutations at high frequencies, like EGFR and KRAS, NGS is of limited use. Unlike Nicole Ezer's study, where EGFR mutations are rare in metastatic lesions, our study found a common occurrence of EGFR mutations in metastatic lesions. A domestic study [17] showed EGFR L858R as the most common mutation in MPLC. In MPLC, EGFR L858R is preferred in cases with a single trunk driver mutation, while PM cases exhibit a higher total number of backbone mutations and higher concordance rates, reflecting the genetic clonal characteristics of tumor cells. Additionally, mutation abundance was significantly higher in intrapulmonary metastasis lesions than in primary tumors, leading to pathologist reconsultation for histologically suggestive metastasis.

Our study has limitations, including a small sample size and a relatively small gene panel. Follow-up survival analyses were not performed, preventing a comparison of overall survival (OS) between patients classified as MPLC and PM by NGS and those predicted by AJCC. Chen et al. [18] demonstrated significant differences in the detection of EGFR mutation abundance between MPLC and PM tumor pairs, supporting the usefulness of NGS in distinguishing between MPLC and PM. In our study, specimens with a tumor cell content $\geq$20 % showed significantly increased EGFR mutation abundance in metastases compared to primary lesions, suggesting gene clonality characteristics and a homologous correlation between the two lesions.

NGS has significant clinical value in lung cancer diagnosis, enabling precise detection of driver gene mutations (e.g., EGFR, ALK, KRAS) and resistance-associated mutations to guide individualized treatment. Additionally, NGS can assess tumor mutation burden (TMB) and microsatellite instability (MSI), aiding immunotherapy decisions. Noninvasive monitoring via ctDNA detection allows dynamic assessment of disease progression and treatment response.

By distinguishing MPLC from PM, this study provides molecular insights to refine clinical decision-making,**Surgical planning:** Sublobar resection is prioritized for MPLC to preserve lung function, whereas PM requires systemic therapy evaluation to minimize overtreatment. **Targeted therapy:** MPLC lesions with divergent driver mutations (e.g., EGFR vs. ALK) may demand lesion-specific agents, while PM lesions sharing drivers benefit from monotherapy. **Staging guidelines:** Integrating molecular independence (e.g., clonal evolution evidence) into TNM staging prevents misclassifying MPLC as advanced PM, thereby improving prognostic accuracy. **Confounding factors and technical limitations, Tumor heterogeneity:** Single-region biopsies risk missing critical molecular features, leading to clonal misinterpretation. **Clonal timing:** Early metastatic lesions may molecularly resemble primary tumors, challenging static sequencing-based distinctions. **Treatment artifacts:** Prior chemoradiotherapy can induce secondary mutations, obscuring driver gene profiling. **NGS constraints:Sensitivity,**Low tumor purity ($<$20 %) or low-frequency mutations (VAF$<$5 %) may evade detection. **Structural variants:** Short-read sequencing (e.g., Illumina) struggles to resolve complex SVs like translocations. **Threshold bias:** Arbitrary cutoffs (e.g., VAF$\geq$5 %) risk excluding true biological signals. **Database gaps:** Rare mutations lack clinical annotations, introducing functional uncertainty. **Study limitations**, **Retrospective design:** Potential selection bias may inflate molecular discriminative power. **Sampling inadequacy:** Sequencing dominant lesions alone risks missing subclonal evolution. **Resource barriers:** NGS implementation remains logistically challenging in resource-limited regions.

In the future, NGS will advance toward higher sensitivity, lower costs, and faster turnaround times. Integration with single-cell sequencing, spatial omics, and AI-driven bioinformatics will enhance the resolution of lung cancer heterogeneity. Furthermore, multi-omics approaches (genomics, transcriptomics, proteomics) are expected to identify novel biomarkers and provide a more comprehensive foundation for personalized precision therapy.

While the gold standard for lung cancer staging remains histopathological examination, NGS predicts the clonal relationship between primary and metastatic lesions, aligning with AJCC criteria and clinical outcomes. In cases of ambiguous pathology and imaging diagnoses, NGS provides a convenient adjunct to traditional findings, aiding accurate postoperative targeted drug or immunotherapy guidance.

However, NGS testing remains limited, primarily due to the high genetic heterogeneity of tumors. Multiple lesions in the same patient may develop similar mutation patterns over time, making it challenging to distinguish MPLC from PM. Additionally, MPLC and PM may share driver mutations (e.g., EGFR, TP53), complicating differentiation based solely on mutation profiles. NGS relies on tumor tissue or ctDNA, and the representativeness and abundance of samples can impact mutation detection accuracy. Furthermore, somatic mutations may arise independently in different tumor lesions, while certain germline mutations can mislead results. Current bioinformatics analysis methods still struggle to fully distinguish MPLC from PM, making it difficult to eliminate false positives and false negatives. In summary, future research should focus on enhancing the accuracy and efficiency of NGS technologies, refining bioinformatics tools and databases, and driving continuous innovation and application of NGS in NSCLC.

CRediT authorship contribution statement

Ming Cheng: Writing – original draft, Writing – review & editing. **Shujun Shao:** Conceptualization, Writing – original draft. **Wei Xu:** Data curation, Formal analysis. **Dazhi Liu:** Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Writing – review & editing.

Reporting checklist

The authors have completed the STROBE reporting checklist.

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 conducted in accordance with the Declaration of Helsinki (as revised in 2013). This study was approved by the ethics board of General Hospital of Northern Theater Command (NO. Y2023-011) and individual consent for this retrospective analysis was waived.

Data availability statement

The data are available from the corresponding author on reasonable request.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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