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The potential role of next-generation sequencing in identifying *MET* amplification and disclosing resistance mechanisms in NSCLC patients with osimertinib resistance

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Purposes: Osimertinib, one of the third-generation *EGFR*-tyrosine kinase inhibitors (TKIs) designed to target *EGFR* T790M mutation, significantly improves the prognosis of lung cancer. However, drug resistance still happens and *MET* amplification is responsible for one of the main causes. Fluorescence *in situ* hybridization (FISH) is the gold standard for *MET* amplification detection, but fundamentally limited by observer subjectivity. Herein, we assessed the value of next-generation sequencing (NGS) method in *MET* amplification detection in non-small cell lung cancer (NSCLC), as well as revealed the mutation profiling of NSCLC patients with osimertinib resistance to provide some valuable clues to the mechanisms of resistance.

Methods: A total of 317 cancer tissue samples from 317 NSCLC patients at time of progression following osimertinib were submitted to NGS and only 96 tissues were tested by FISH simultaneously. With FISH results as gold standard, enumeration algorithm was applied to establish the optimal model for identifying *MET* amplification using gene copy number (GCN) data.

Results: The optimal model for identifying *MET* amplification was constructed based on the GCN of *MET*, *BRAF*, *CDK6* and *CYP3A4*, which achieved a 74.0% overall agreement with FISH and performed well in identifying *MET* amplification except polysomy with a sensitivity of 85.7% and a specificity of 93.9%. The inconsistency between NGS and FISH occurred mainly in polysomy subtype, while *MET* GCN ≥ 5 could be reliably recognized by NGS. Moreover, the most frequently mutated genes in NSCLC patients with osimertinib resistance were *EGFR* (59.94%), followed by *TP53* (43.85%), *NRG1* (9.46%), *PIK3CA* (6.31%), and ATM (5.36%). The known resistance mechanisms, including *MET* amplification, *EGFR* (C797S, L718Q/R), *TP53*, *CDK4*, *CDK6*, *CDKN2A*, *BRAF*, *KRAS*, *NRAS* and *PIK3CA* mutations were also disclosed in our cohort.

Conclusions: NGS assay can achieve a high concordance with FISH in *MET* amplification detection and has advantages in portraying various genetic alterations, which is of worthy in clinical promotion.

KEYWORDS

next generation sequencing, non-small cell lung cancer, MET amplification, osimertinib resistance, fish

1 Introduction

Lung cancer is the leading cause of cancer-related mortality worldwide. Non–small cell lung carcinoma (NSCLC) constitutes approximately 85% of all the lung cancers and has a poor 5-year survival rate of ~20% (1, 2), despite great efforts made over the past decades. The development of epidermal growth factor receptor (*EGFR*)-tyrosine kinase inhibitors (TKIs) is an important milestone in the targeted therapy of NSCLC (3). Numerous clinical trials have demonstrated that both the first-generation *EGFR*-TKIs such as gefitinib and erlotinib, and the second-generation *EGFR*-TKIs represented by afatinib achieved superior efficacy in the treatment of the *EGFR*-mutant NSCLC patients (4, 5). However, most NSCLC patients develop drug resistance, with *EGFR* T790M mutation as the most common resistant mechanism (6). To overcome the T790M-mediated resistance, the third-generation *EGFR*-TKIs, osimertinib, targeting the T790M mutation emerged as the times require. However, patients also inevitably develop resistance, which limits the long-term efficacy of third-generation *EGFR*-TKIs in the clinic (7, 8). Of note, osimertinib has been recommended as the preferred first-line treatment option for *EGFR*-mutated NSCLC at present (9). Therefore, it is necessary to comprehensively explore the resistance mechanisms of osimertinib.

Mesenchymal-epithelial transition (*MET*) gene located on chromosome 7 (7q31) encodes the receptor tyrosine kinase or hepatocyte growth factor receptor (HGFR). HGFR, along with its ligand, HGF, functions as an important regulator of cell survival, proliferation, motility and migration (10, 11). Dysregulation of *MET* signaling, such as *MET* amplification, *MET* exon 14 skipping mutation, and *MET* overexpression has been found to be associated with the development of lung cancer (12). *MET* amplification referring to the *MET* gene copy number (GCN) gains occurs in 1-6% of newly diagnosed NSCLC patients and is considered as a poor prognostic marker (13, 14). Moreover, growing evidence has demonstrated that *MET* amplification is a key driver of acquired resistance to *EGFR*-TKIs in addition to *EGFR* mutation, especially for patients resistant to the third-generation of *EGFR*-TKIs (15). Detailly, the incidence of *MET* amplification in NSCLC patients resistant to third-generation *EGFR*-TKIs increased from 5-22% to 5-50% compared to the patients' resistance to the first/second-generation *EGFR*-TKIs (16). Of note, some clinical studies have demonstrated that the combination therapies of *EGFR*-TKIs and *MET* inhibitors improved the outcomes of NSCLC patients with *MET* amplification (17, 18). Therefore, accurate detection of *MET* amplification is essential for NSCLC patients with *MET* amplification.

To date, various techniques, including fluorescence *in situ* hybridization (FISH), quantitative real-time polymerase chain reaction (qRT-PCR) and next-generation sequencing (NGS), have been developed for detecting *MET* GCN (19). Both polysomy (multiple copies of chromosome 7) and *MET* amplification (multiple copies of *MET* only) cause *MET* GCN gains. FISH is the gold standard for detecting *MET* amplification status, but fundamentally limited by reliance on the subjectivity of the observers (20). NGS is increasingly applied in clinical practice for the detection of *MET* GCN, which offers comprehensive profiling not only *MET* amplification, but also many other genetic alterations that cannot be detected by FISH but are of high clinical significance. To date, several studies have explored the consistency between NGS and FISH for *MET* amplification detection. Unfortunately, the concordance between the FISH and NGS was no more than 70% (21, 22). Also, no consensus on the definition of *MET* amplification is reached, with the cutoff values ranging from GCN 2.3-10 depending on different NGS platforms (16, 22). In addition, *MET* amplification is regarded as a truly oncogenic driver compared to polysomy (23, 24). Therefore, it is necessary to find out a standardized NGS method to effectively define *MET* amplification.

In our study, we mainly investigated whether NGS could serve as an alternate method to identify *MET* amplification status, especially *MET* amplification, and reported the mutation profiling of the largest cohort of Chinese NSCLC patients with osimertinib resistance to reveal the underlying mechanisms to resistance. To this end, we first constructed the optimal model to identify *MET* amplification based on the GCN of *MET* and other genes located on chromosome 7 in 96 NSCLC patients, with FISH results as the gold standard. Then, we described the genetic mutation profile of 317 patients with resistance to osimertinib, and explored the relationship between genetic mutation and *MET* amplification status. Finally, we disclosed the known mechanisms of resistance to osimertinib in our cohort. Figure 1 showed the experimental flowchart of this study.

2 Method

2.1 Patients and samples

A total of 317 NSCLC patients from Shanghai Chest Hospital between August 2021 to October 2022 were enrolled in this study. All cases received first- and/or second-generation *EGFR*-TKIs as the first-line treatment, followed by third-generation *EGFR*-TKIs (osimertinib) as the second-line treatment, and eventually showed drug resistance. The tissue samples from 317 NSCLC patients at the time of progression following osimertinib were collected for genetic abnormality analysis, of which all cases were analyzed by NGS and only 96 by FISH simultaneously. Therefore, 96 NSCLC cases could be directly compared using both NGS and FISH regarding the determination of the *MET* amplification status. Clinic data including the age, gender, stage, and histology type were collected from the medical records. Informed consents were obtained from all patients and this study was carried out in compliance with the Declaration of Helsinki and approved by the ethics committee of Shanghai Chest Hospital (No. KS(Y)22288).

2.2 Fluorescent *in situ* hybridization

FISH was performed on 4-μm-thick formalin-fixed, paraffin-embedded (FFPE) tissue sections using a *MET*/*CEP7* (centromere of chromosome 7) dual-color FISH probe (Vysis, Abbott Laboratories, Illinois, USA) according to the manufacturer's instructions. The mean copy number of *MET* and *CEP7* was recorded in at least 50 non-overlapping tumor cell nuclei and then *MET*/*CEP7* ratio was calculated. *MET* amplification was defined as a mean *MET*/*CEP7* ratio ≥ 2, and polysomy was defined as a mean GCN ≥ 5 and *MET*/CEP7 ratio < 2 synchronously (25).

2.3 Next-generation sequencing

Genomic DNA (gDNA) was extracted from FFPE tissues using the QIAamp FFPE DNA Tissue Kit (Qiagen, Germantown, MD, USA). In general, 200 ng gDNA was used to create the sequencing library targeting 84 genes (listed in Supplementary Table 1) (Rightongene, Shanghai, China) using a custom hybrid-capture NGS panel. Briefly, the DNA was fragmented and the fragmented DNA was subjected to end-repairing, A-tailing, and ligation with indexed adapters. Then, the libraries were PCR-amplified and purified for target enrichment. The library concentration was recorded by Qubit 3.0 Flourometer (Thermo, Massachusetts, USA) and the length and purity of the library fragment were measured by Qsep100 automated nucleic acid protein analysis system (BIOptic, Jiangsu, China). 500 ng indexed DNA libraries were pooled to obtain 1.5 μg DNA. Pooled DNA samples were mixed with DNA blocker and dried in a concentrator. Hybridization Master Mix was added to each sample. The mixtures were incubated at 95 °C for 10 min, then combined with probes and incubated at 65 °C for 16 h. Target regions were captured in accordance with the manufacturer's instructions. The concentration and fragment size distribution of the final library were determined using Qubit 3.0 fluorometer and Qsep100 automated nucleic acid protein analysis system, respectively. Targeted NGS was performed on the Novaseq platform (Illumina, California, USA). The NGS detection sensitivity was approximately 0.1%.

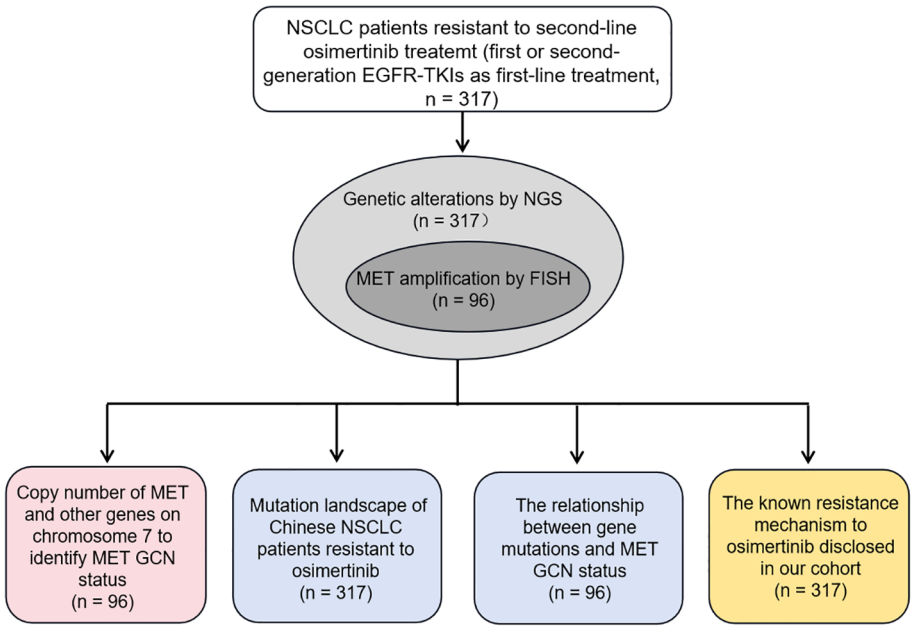

FIGURE 1
The experimental flowchart of this study.

2.4 Bioinformatic analysis

Original sequencing image data file was converted into the sequencing data through base recognition and stored as FASTQ file. The quality of the original sequencing data was evaluated by FastQC (version 1.11.4) software. Trimmatic (version 3.6) software was used to remove joint information, low-quality bases, or undetected bases. After sorting and eliminating repetitive sequences, BAM files were obtained. The data of BAM files including library average length, comparison rate, coverage, capture rate, sequencing depth, homogeneity was used to evaluate the quality of sample library. Based on the BAM results, the SNP and InDel sites were detected by GATK and Mutet2 (26, 27) and annotated by ANNOVAR (28). Germline variants were removed using the ExAC (29), and 1000 Genomes project (30) (>0.1% population frequency). In addition, mutation meeting the following criteria were filtered out: (1) variant allele frequency (VAF) of mutation was less than 1%; (2) mutations predicted as harmless mutation by SIFT (SIFT score > 0.05) (31), and Polyphen2 (Polyphen2 HDAV score ≤ 0.446) (32). The final retained variants may be deleterious, likely deleterious or unknown significance. For the gene copy number identification, we first established a baseline of relative read coverage for capture region using normal tissue samples and then compared clinical samples to this baseline. Variant calling and copy-number variation analysis were performed by the cnvkit (https://github.com/etal/cnvkit).

2.5 Construction of the optimal model to identify *MET* amplification

Enumeration algorithm was applied to construct the optimal model to identify *MET* amplification using GCN data in 96 patients with FISH results. Receiver operating characteristic (ROC) curves were used to identify cutoff values of CN (*MET*)/CN (*EGFR*, *BRAF*, *CDK6*, *PMS2*, *ABCB1* and *CYP3A4*, which were all of other genes on the 84-gene panel that were on chromosome 7) distinguishing between *MET* amplification subtype and polysomy/negative subtype. Then, enumeration algorithm was used to find out the optimal combination of CN (*MET*)/CN (other genes on chromosome 7) with the highest consistency with FISH in identifying *MET* amplification. Finally, the ROC curve was used to determine the cutoff value of *MET* GCN distinguishing between polysomy subtype and negative subtype. The sensitivity and specificity for identifying *MET* amplification were calculated as the proportion of *MET* amplification cases identified by NGS among *MET* amplification cases identified by FISH, and the proportion of polysomy or negative cases identified by NGS among polysomy or negative cases identified by FISH, respectively. The sensitivity and specificity for identifying polysomy or negative cases were calculated in the same way.

2.6 Statistical analysis

Statistical analysis was performed using R package (version 4.1.2). Categorical variables were evaluated using the chi-squared test or Fisher's exact test. All tests were two-tailed, $p < 0.05$ was considered to be significantly different. * represents $p < 0.05$ and ** represents $p < 0.01$. Graphs were made in Prism 8, v8.2.0 (GraphPad Software Inc.) and Adobe Illustrator 2021 (Adobe Inc.).

3 Results

3.1 Baseline characteristics

A total of 317 NSCLC patients were enrolled in this study, with a median age of 60 years (range from 32 to 84 years). 135 (42.6%) patients were male and most of patients (94.2%) were diagnosed at an advanced stage of IV. 306 (96.8%) cases were lung adenocarcinoma, 9 (2.8%) cases were squamous cell carcinoma, and 1 (0.3%) case was adenosquamous carcinoma. 96 patients were subjected to FISH testing for detecting MET amplification status, where 14 (14.6%) cases were *MET* amplification, 16 (16.7%) cases were polysomy and 66 (68.8%) cases were negative. A summary of the clinical characteristics of 317 NSCLC patients was presented in Table 1. There was no significant difference in clinical characteristics between patients with MET amplification and those without MET amplification (Table 2).

3.2 The optimal model to identify *MET* amplification by NGS

The commonly accepted criteria for defining *MET* amplification status via FISH was showed in Figure 2A. With FISH results as a reference, we explored the appropriate model to define *MET* amplification status based on the GCN of *MET* and other genes (*EGFR*, *BRAF*, *CDK6*, *PMS2*, *ABCB1* and *CYP3A4*) located on chromosome 7. The optimal model demonstrated that cases with *MET*/*CYP3A4* ≥ 1.12, *MET*/*CDK6* ≥ 1.20 and *MET*/*BRAF* ≥ 1.53 were predicted to be *MET* amplification. Among the other cases, the cases with *MET* GCN ≥ 2.8 were predicted to be polysomy; and cases with *MET* GCN < 2.8 were predicted to be negative (Figure 2B). The overall concordance between NGS and FISH was 74.0% (95% CI 64% - 82.4%), while the sensitivity/specificity for identifying *MET* amplification, polysomy and *MET* negative were 85.7%/93.9%, 37.5%/85% and 80.3%/73.3%, respectively (Figure 2C). The model was stronger in identifying *MET* amplification and negative with a sensitivity of 85.7% and 80.3%, respectively; and weaker in identifying polysomy with a sensitivity of 37.5%. When combining polysomy and negative into one group, the overall concordance between NGS and FISH reached 93.9% (Figure 2D), suggesting that polysomy might be the key factor contributing to the discrepancy between NGS and FISH in the detection of *MET* amplification status.

Next, we investigated whether the concordance between NGS and FISH might be mediated by *MET* GCN levels. The distribution of *MET* GCN in all cases ranged from 1.2 to 16.7. The *MET* GCN of 25 discordant cases between NGS and FISH was predominantly distributed between 2 and 5, and the NGS method showed a 100% concordance with FISH when *MET* GCN was ≥ 5 (Figure 2E).

Overall, our optimal model performed well in identifying *MET* amplification, especially the high level amplification.

3.3 Landscapes of genetic mutations in patients resistant to third generation TKIs

The targeted sequencing was performed in all the patients and the mutation landscape of NSCLC patients resistant to osimertinib was showed in Figure 3A. A total of 66 mutated genes and 488 mutation sites were detected in 82.33% (261 of 317) of the NSCLC patients, among which the most frequently mutated genes were *EGFR* (59.94%), followed by *TP53* (43.85%), *NRG1* (9.46%), *PIK3CA* (6.31%), *ATM* (5.36%), *APC* (4.42%), *ARID1A* (4.42%), *BRAF* (4.10%), *NTRK1* (4.10%), *POLE* (4.10%) and *KRAS* (3.79%). Those mutations had multiple mutation forms (missense, nonsense, nonstop, frameshift/inframe and splicing mutations) and mutation types (C→T, C→A, C→G, T→C, T→A and T→G), where the most commonly mutation form and type were missense mutations (424, 62.%) and C→T transitions (260, 41.1%), respectively (Figure 3B). Among the *EGFR* mutant patients, 95.79% of the patients harbored the reported hotspots, including exon 19 deletion (48.42%), L858R (46.84%) and T790M (17.89%), respectively. Moreover, uncommon mutation sites such as A750P (2.63%), C797S (2.11%), E709K (1.05%) accounted for 20.59% of *EGFR* mutations (Figure 3C). The majority of patients (65.8%) possessed single *EGFR* mutation site, a decent number of patients (26.3%) had two *EGFR* mutation sites, and the minority of patients (7.9%) had more than two *EGFR* mutation sites (Figure 3D). These mutational analyses may provide some valuable clues to the mechanisms of osimertinib resistance.

TABLE 1 Clinical characteristics of 317 patients with NSCLC.

Variables	Number of patients (%)
Age (years)	
Median	60
Range	32-84
Gender	
Male	135 (42.6)
Female	182 (57.4)
Clinical stage	
III	6 (5.8)
IV	97 (94.2)
NA	214
Histology type	
LUAD	306 (96.8)
LUSC	9 (2.8)
ASC	1 (0.3)
NA	1
MET amplification status identified by FISH	
MET amplification	14 (14.6)
Polysomy	16 (16.7)
Negative	66 (68.8)
NA	221

LUAD, lung adenocarcinoma; LUSC, lung squamous cell carcinoma; ASC, adenosquamous carcinoma.

3.4 Correlations between genetic mutations and *MET* amplification status

Then, we displayed the mutation landscapes of NSCLC patients with *MET* amplification, polysomy and *MET* negative, respectively (Figure 4A), and explored the correlations between genetic mutations and *MET* amplification status. The results showed that frequencies of mutations such as *EGFR* L858R (50.0% vs. 68.8% vs. 25.8%, $p = 0.004$), *ARID1A* (7.1% vs. 25.0% vs. 4.5%, $p = 0.033$), *ATM* (21.4% vs. 0% vs. 3.0%, $p = 0.045$), *NARS* (0% vs. 12.5% vs. 0%, $p = 0.046$) were differed among *MET* amplification, polysomy and *MET* negative groups. After multiple comparisons, we found that NSCLC patients with polysomy had a significantly higher incidence of *EGFR* L858R ($p = 0.002$), *ARID1A* ($p = 0.025$) and *NRAS* ($p = 0.036$) and significantly lower incidence of *EGFR* del19 ($p = 0.041$) mutation compared with those with *MET* negative. Moreover, mutation in *ATM* ($p = 0.035$) was significantly more common in NSCLC patients with *MET* amplification compared with those with *MET* negative. In addition, no association was found between other mutations and *MET* amplification status (Figure 4B). These findings suggested that there may be a potential link between *MET* amplification status and gene mutations.

TABLE 2 Differences in clinical characteristics between patients with *MET* amplification and those without *MET* amplification.

Variables	Patients with *MET* amplification by FISH (n=14)	Patients without *MET* amplification by FISH (n=82)	*P* value
Age (years)			0.848
Median	59.5	59.5	
Range	33-77	41-82	
Gender			1.000
Male	7 (50)	43 (52.4)	
Female	7 (50)	39 (47.6)	
Clinical stage			1.000
III	1 (7.1)	5 (6.1)	
IV	13 (92.9)	77 (93.9)	
Histology type			0.554
LUAD	13 (92.9)	78 (95.1)	
LUSC	1 (7.1)	3 (3.6)	
ASC	0 (0)	1 (1.2)	

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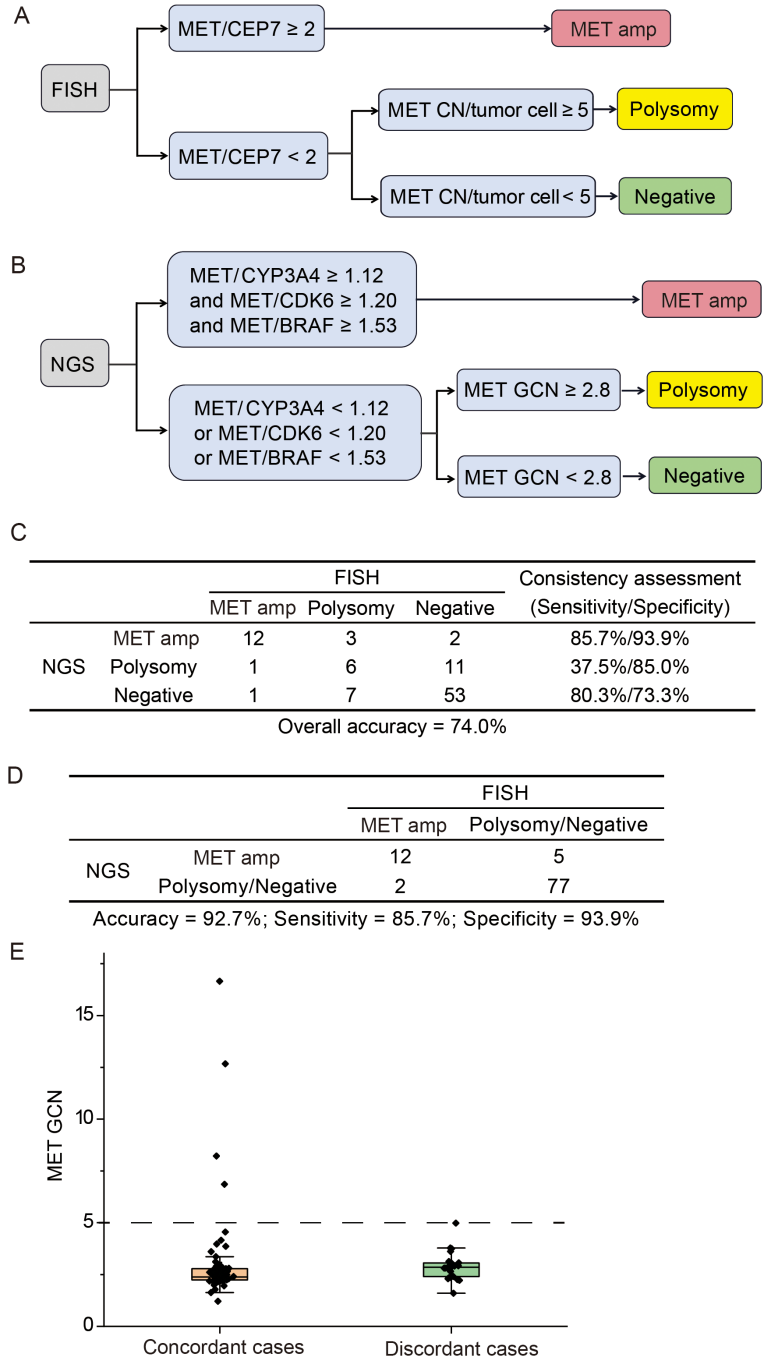

FIGURE 2
Comparison of NGS and FISH for detecting *MET* gene copy number status. **(A)** The criteria for defining *MET* amplification status via fluorescence *in situ* hybridization (FISH). **(B)** The optimal model for identifying *MET* amplification status via next-generation sequencing (NGS). **(C)** The concordance between NGS and FISH in identifying *MET* amplification, polysomy and negative. **(D)** The consistency between NGS and FISH in identifying *MET* amplification vs. other cases. **(E)** Distribution of *MET* gene copy number for concordant and discordant cases. Amp, amplification; GCN, gene copy number.

3.5 The known osimertinib resistance mechanisms disclosed by NGS in our cohort

Figure 5 summarized the known genetic alterations associated with osimertinib resistance identified in our study. It is well recognized that the osimertinib resistance mechanisms are broadly divided into *EGFR*-dependent (on-target) and *EGFR*-independent (off-target) mechanisms. In our cohort, the known on-target mutations in C797S (1.26%), L718Q/R (0.95%), G796S (0.32%), G724S (0.32%), G719X (0.32%) sites were identified in several patients (33). Regarding *EGFR*-independent mechanisms, the known off-target alterations, such as *MET* amplification (14 of 96, 14.58%, bypass signaling activation), *TP53* mutation (43.85%)

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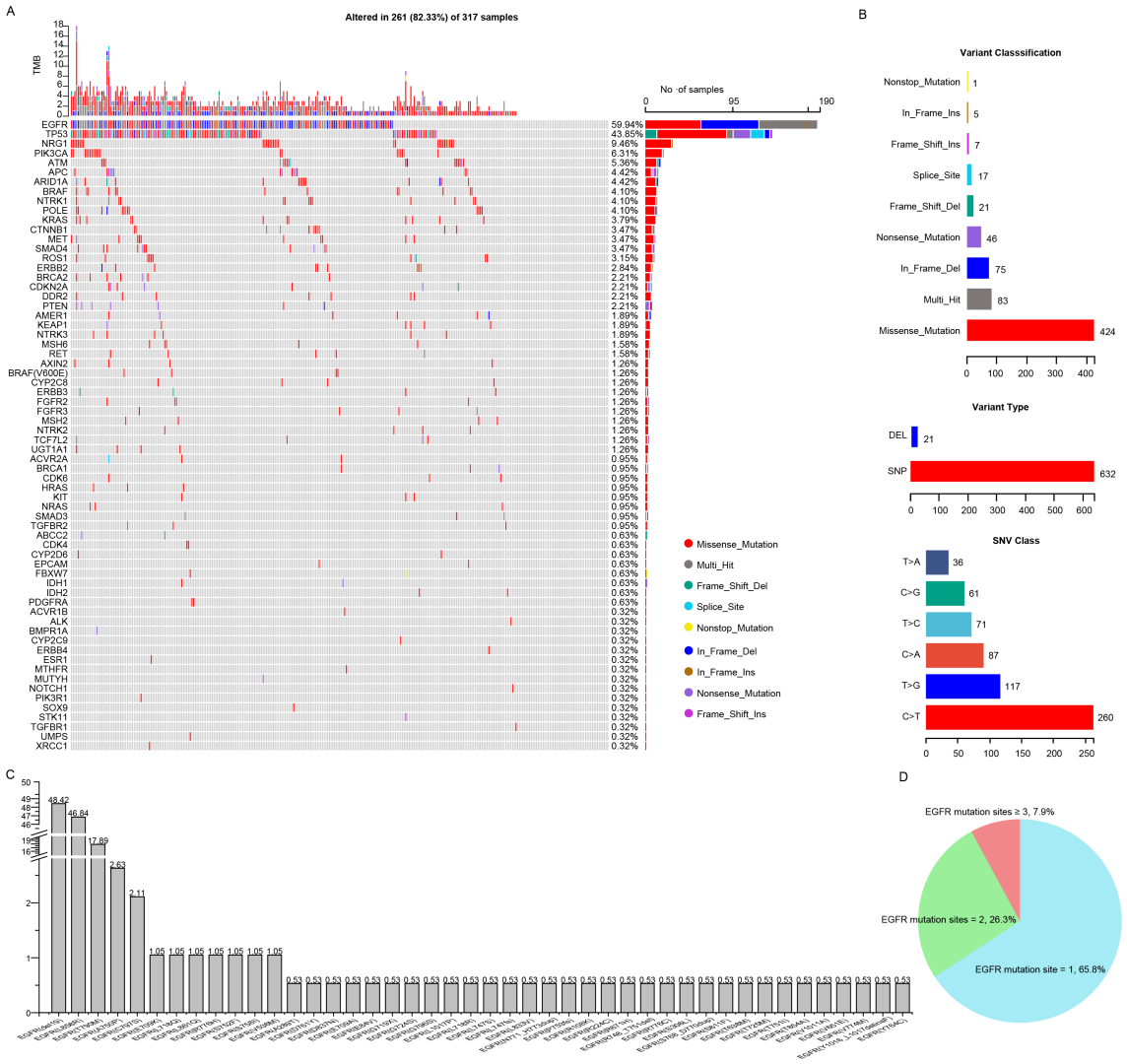

FIGURE 3
Comprehensive mutation analysis of Chinese NSCLC patients with osimertinib resistance. **(A)** Gene mutational landscapes of NSCLC patients with osimertinib resistance. **(B)** Classifications of mutation forms and mutation types in NSCLC patients with osimertinib resistance. **(C)** Bar chart showing the mutation frequencies of *EGFR* mutation sites. **(D)** Pie chart showing the percentage of patients carrying different numbers of *EGFR* mutation sites.

(34, 35), *PIK3CA* mutation (6.31%, PI3K/AKT/PTEN/mTOR pathway activation) (33), *BRAF*/*KRAS*/*NRAS* mutation (5.36%/3.79%/0.95%, RAS-RAF-MEK-MAPK pathway activation) (36), and *CDK4*/*CDK6*/*CDKN2A* mutation (0.63%/0.95%/2.21%, cell cycle gene) (37, 38), were observed in 52.05% of the current cohort.

4 Discussion

The use of reliable methods for the detection of *MET* amplification status in NSCLC is essential for identifying patients eligible for treatment with *MET* inhibitors. In the present study, the *MET* amplification status identified by the optimal model demonstrated excellent agreement with the FISH results, suggesting that NGS may be an alternative method for *MET* amplification detection. In addition, our study reported the comprehensive mutation profile of a largest cohort of Chinese NSCLC patients with osimertinib resistance, which may contribute to the discovery of potential resistance mechanisms and the development of new *EGFR*-TKIs.

In recent years, NGS has been widely applied in clinical practice for the detection of comprehensive gene profiles, including gene mutations, gene amplification, rearrangement, and fusion. However, it is still lacking robust evidence regarding the feasibility and appropriateness of NGS as an alternative method to FISH to identify *MET* amplification. To date, several studies have investigated the performance of NGS in *MET* amplification detection using FISH as the gold standard (16, 39). Unfortunately, the concordance between the FISH and NGS in

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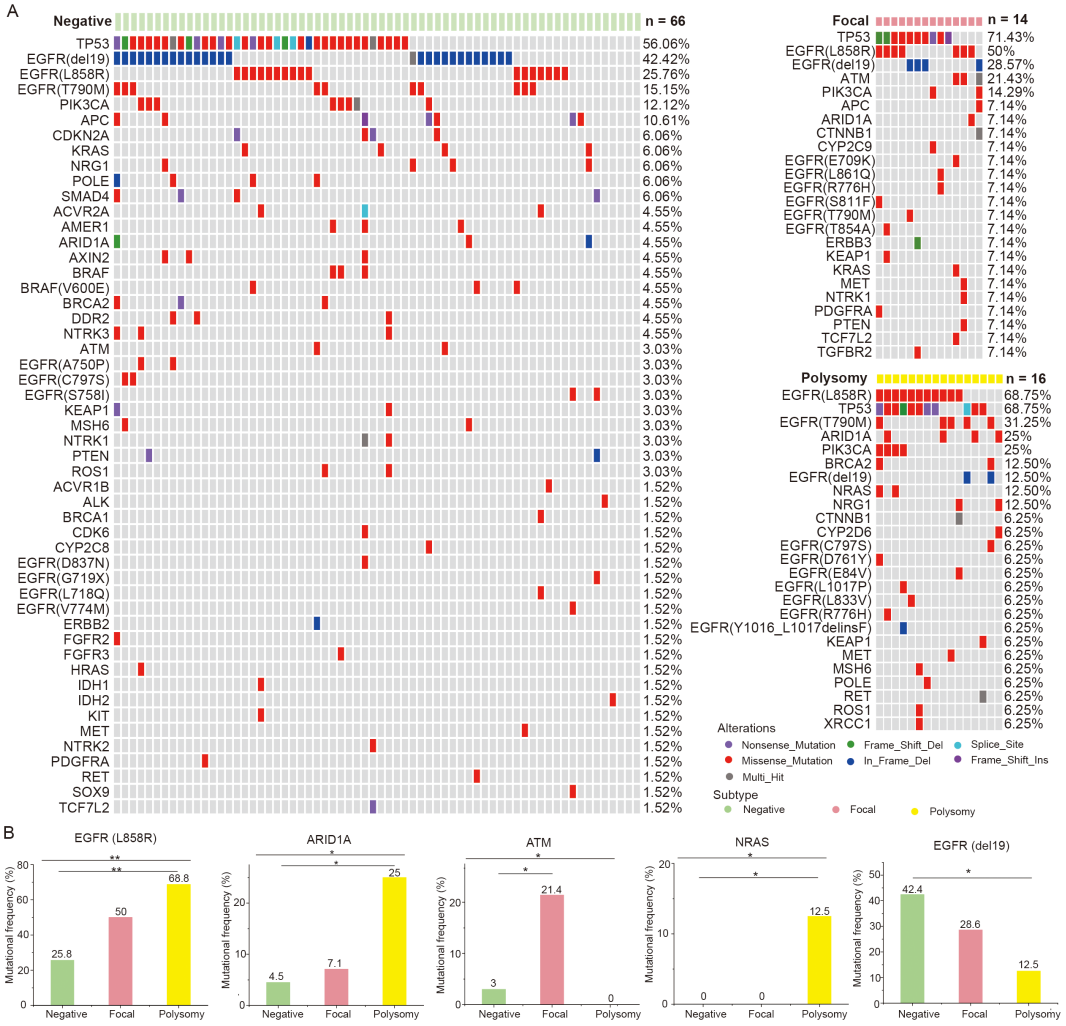

FIGURE 4
Correlations between genetic mutations and *MET* amplification status. **(A)** The gene mutational landscapes of NSCLC patients with *MET* amplification, polysomy and *MET* negative. **(B)** Genes with significantly different mutation frequencies in *MET* amplification, polysomy and *MET* negative groups. Amp, amplification. * represents $p < 0.05$ and ** represents $p < 0.01$.

identifying *MET* amplification status was low. For example, Lai et al. (21) reported the low correlation of *MET* amplification results obtained by NGS and FISH, where of the 18 NSCLC patients identified as FISH-positive (2 *MET* amplification and 16 polysomy), only 8 (44.4%) were deemed to have *MET* GCN gain according to NGS. Also, the TATTON study reported that among 47 FISH-positive patients, only 12 (27%) were diagnosed with *MET* amplification by NGS (40). In addition, the concordance rate among NGS and FISH was only 62.5% (25 of 40) in the study performed by Peng et al. (22). In our study, the optimal model in identifying *MET* amplification status achieved a relatively high concordance rate of 74.0% with FISH for detecting *MET* amplification status.

Notably, accumulated evidences have suggested that the *MET* amplification is the truly oncogenic driver of lung cancer. NSCLC patients with *MET* amplification presented a more robust response to *MET* inhibitors compared with those with polysomy in the published results of clinical trials (41, 42). Our study prioritized the identification of *MET* amplification. In terms of the identification of *MET* amplification, our NGS method achieved a sensitivity of 85.7% with FISH, providing a reliable measurement of this biomarker in the clinic. In contrast to previous studies, which only used single gene as the determinant to distinguish *MET* amplification from polysomy (16, 21), we utilized *CYP3A4*, *CDK6*, and *BRAF* genes to determine *MET* amplification, which allowed for its potential higher resolution in the discrimination of *MET* amplification status. Moreover, previous studies indicated that high-level amplified *MET* has been used as a biomarker to predict the benefit of *MET* inhibitors, emphasizing the importance of identifying high levels of *MET* amplification (43, 44). In our study, a 100% consistency rate between NGS and FISH was observed in samples harboring *MET* GCN ≥ 5, which indicated that high-level amplified *MET* samples can be reliably detected via NGS. This finding was consistent with the previous study performed by Schubart et al. (43), who reported that the NGS method showed the best concordance with FISH when *MET* GCN was > 10 (80%, 4 of 5). Interestingly, Xiang et al. (41) found that IHC (H-score ≥ 200) showed high overall consistency with FISH in identifying *MET* amplification. Hartmaier et al. (45) reported that

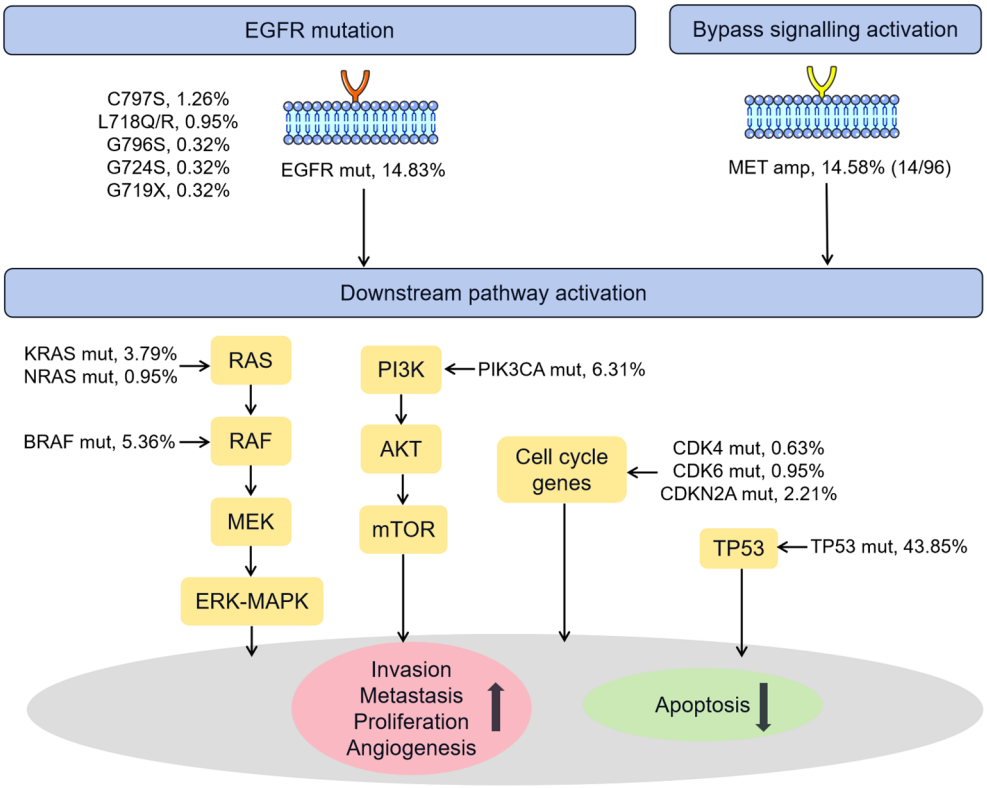

FIGURE 5
Schematic representation of the known mechanisms of resistance to osimertinib identified in our cohort. Amp, amplification; Mut, mutation.

81.25% (13 of 16) cases of *MET* amplification by FISH were also IHC positive, and IHC expression tended to increase with increasing *MET* GCN (≥10) in FISH-positive tumors. These studies indicated the potential relationship between *MET* amplification by FISH and *MET* IHC. It would be of interest to look at the correlation of *MET* amplification by NGS with *MET* IHC in the future.

Over the past few decades, genetic alterations of cancer driver genes have been identified in NSCLC, and molecular testing and targeted therapies have become standard care for NSCLC patients (46). Osimertinib is currently the preferred first-line therapy in patients with NSCLC with common *EGFR* mutation and the standard second-line therapy in T790M-positive patients in progression to previous *EGFR*-TKIs (47, 48). Osimertinib is a highly effective treatment with a high response rate and long-lasting disease control. However, the resistance to the treatment inevitably develops among patients (38, 49). Therefore, a comprehensive understanding of genetic alterations in osimertinib resistant patients is crucial to characterize emerging molecular resistance mechanism and develop novel targeted treatment. Our study presented comprehensive mutation profile of a largest cohort of Chinese NSCLC patients with osimertinib resistance, which may provide some clinically valuable clues to understand the mechanism of osimertinib resistance. To date, a number of studies have revealed that resistance mechanisms to osimertinib are highly complex, including *EGFR*-dependent and *EGFR*-independent mechanisms (8, 48, 50). The *EGFR*-dependent mechanisms include *EGFR* mutations or amplifications, where the most common *EGFR* mutation is *EGFR* C797S, accounts for 0-29% of cases of resistance to osimertinib (48). In our study, the incidence of C797S mutations was 1.26%. Besides C797S mutations, several other known on-target mutations in L718Q/R (0.95%), G796S (0.32%), G724S (0.32%), G719X (0.32%) were also identified. Regarding *EGFR*-independent mechanisms, it has been reported that osimertinib resistance can be acquired by bypass pathway activation, downstream pathway activation, cell cycle gene mutation, and histologic transformation (33, 35, 36, 51). In our study, the off-target alterations were dominant, mainly in *TP53* mutation and *MET* amplification. It is worth noting that besides the discovery of the known genetic alterations medicating osimertinib resistance, a number of on-target mutations such as *EGFR* A750P (1.58%), E709K (0.63%), L861Q (0.63%), R776 (0.63%), S752F (0.63%), S758I (0.63%), and V536M as well as off-target mutations such as *NRG1* (9.46%), *ATM* (5.36%), *APC* (4.42%), *ARID1A* (4.42%), *NTRK1* (4.10%), *POLE* (4.10%), were also identified in multiple patients in our cohort. This finding may provide important guidance for future oncology efforts, such as exploring the potential resistance mechanisms and developing new *EGFR*-TKIs or combined strategies for NSCLC patients.

Several limitations of this study should be stated. First, regarding the comparison of FISH and NGS results, this study involved a relatively small sample size and lacked a validation cohort, which may affect the generalizability of our NGS methods for identifying *MET* amplification status. Second, no treatment response and prognosis data were available in this study. Further research is needed to validate the clinical utility of our NGS methods in determining

tumors with *MET* amplification. We intend to carry out a larger sample size study to further validate our finding and examine the clinical utility of *MET* amplification by our NGS methods.

Collectively, this study demonstrated the potential of NGS as an alternative method in identifying *MET* amplification. In addition to *MET* amplification, DNA-based NGS assay could provide other data, such as characterizing various genetic alterations, which may potentially serve as an effective tool for guiding therapeutic strategies.

Data availability statement

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Ethics statement

The study was carried out based on the Declaration of Helsinki and approved by the Ethics Committee of Shanghai Chest Hospital with No. KS(Y)22288.

Author contributions

XX: Conceptualization, Investigation, Writing – original draft. RX: Investigation, Writing – review & editing. JL: Investigation, Writing – review & editing. BX: Conceptualization, Writing – original draft. CW: Methodology, Software, Writing – review & editing. KZ: Software, Writing – original draft. HZ: Conceptualization, Writing – review & editing. XC: Conceptualization, Writing – review & editing.

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Conflict of interest

Author XX, RX, BX, CW, and KZ were employed by the company Shanghai Rightongene Biotechnology Co., Ltd.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fonc.2024.1470827/full#supplementary-material

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