Comprehensive Analysis of NGS and ARMS-PCR for Detecting EGFR mutations Based on 4467 Cases of NSCLC Patients

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Research

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Abstract

Background

By comparing the detection rate and type of targeted gene mutations in non-small cell lung cancer (NSCLC) between amplification refractory mutation system PCR (ARMS-PCR) and next generation sequencing (NGS), the characteristics and application advantages of non-small cell lung cancer detection are explained, providing a basis for clinicians to effectively select the corresponding detection methods.

Methods and materials

The cases of targeted genes for lung cancer were selected from the First Affiliated Hospital of Chongqing Medical University from January 2016 to October 2020. A sample of 4467 cases was selected and they were diagnosed with NSCLC by Pathological biopsy. Sample sources include surgical resection, bronchoscope biopsy, metastatic biopsy, blood, sputum, cytology of pleural effusion. Among them, 3665 cases were detected by ARMS-PCR technique, and 802 cases were detected by NGS technology. The detection rate and type of ARMS-PCR and NGS techniques for EGFR gene mutations (including exon 18, exon 19, exon 20, exon 21 and so on) in different NSCLC samples were compared respectively.

Results

The total mutation rate of EGFR gene detected by ARMS-PCR was 47.6% while 42.4% detected by NGS which indicated that there was a significant difference between the two methods in detecting total mutation of EGFR gene (P<0.001). In different exons, the EGFR mutation rate detected by two methods is various. The mutation rate of exon 19 by ARMS-PCR detection was evidently higher than that of NGS detection, while the mutation rate of exon 20 and 21 by ARMS-PCR detection were statistically significantly lower than that of NGS detection. Moreover, the multiple mutation rate detected by NGS was 16.3% which was much higher than the 2.7% detected by ARMS-PCR with statistically different.

Conclusions

It showed that NGS could direct the drug use for the resistant patients. However, some rare loci could be detected by NGS but the importance and directed meaning are still unknown and the number of rare mutations is rare too. Further research on new biomarkers and technique is still needed for early diagnosis, directing drug use and assessing the therapy prognosis.

Background

Lung cancer is the most common malignancy worldwide as well as the leading cause of cancer death in both sexes all around the world with 1.8 million new cases and 1.6 million deaths in 2012, and the incidence and mortality of lung cancer have been increasing year by year. In China, lung cancer is the most common cancer among men and second in women, while the disease has the highest death rate among all cancer. According to the degree of differentiation and morphological characteristics of lung cancer cells, lung cancer can be classified into two categories: non-small cell lung cancer (NSCLC) and small-cell lung
cancer (SCLC), with the former accounting for about 85% of diagnosed lung cancers\(^4\). NSCLC is the highly malignant tumor, and most patients were at an advanced stage when they had been first diagnosed. The surgical treatment, chemotherapy and radiotherapy were the common treatment methods at some time in the past, however, the chemotherapy and radiotherapy had limited efficacy and did not maximize the benefits of patients. At present, the targeted drug therapy has been applied to the treatment of NSCLC, and has shown good therapeutic effects\(^5\). The in-depth research and clinical use of epidermal growth factor receptor (EGFR) and epidermal growth factor receptor-tyrosine kinase inhibitor (EGFR-TKI) have provided a dawn for the treatment of advanced NSCLC. Patients with EGFR gene mutation are more sensitive to EGFR-TKI\(^6,7\). Therefore, the detection of EGFR mutation has become a predictive method in targeted treatment of lung cancer\(^8\). In China, the relationship between the mutation rate, distribution of mutation points of EGFR and clinicopathological characteristics is different in diverse regions\(^9\). So far, there is no clinical data onto the relationship between EGFR mutations and clinical pathological characteristics in NSCLC patients in Southwest of China.

At present, the amplification refractory system-PCR (ARMS-PCR) and next generation sequencing (NGS) technology are clinically commonly detection methods which were used to detect the targeted gene mutation. There was high sensitivity and specificity in ARMS-PCR technology, and the detection rate of gene mutation was far exceeding the traditional PCR, had become one of the most popular and important techniques in the personalized molecular detection of tumors\(^10\). On the other hand, NGS, which was based on the principle of edge synthesis edge sequencing, had the advantages of high flux and diverse detection types, and was increasingly used in clinical lung cancer for targeted gene testing. NCCN had recommended that the using of ARMS-PCR or NGS technology for gene mutation detection should prior to targeted treatment of clinical non-small cell lung cancer. In different scenarios, how to choose the right technology of gene testing is a common clinical problem.

In this research, we have analyzed the clinical pathological characteristics and EGFR gene mutations of 4467 patients with NSCLC in Southwest of China, and then analyzed the correlation between the clinical pathological characteristics and EGFR gene mutations which could help to provide data support for targeted drug use. According to the results of targeted gene testing samples, we have compared the advantages and disadvantages of ARMS-PCR and NGS technology which could help clinicians to select an appropriate gene testing method in order to choose the right targeted drug for the lung cancer patients effectively.

### Materials And Methods

#### Patients and tumor tissue samples

Totally, 4467 NSCLC patients’ sample data has been collected who were examined and confirmed NSCLC by histopathology, from January 2016 to October 2020 (4 years and 10 months), in The Frist Affiliated Hospital of Chongqing Medical University. The histopathological diagnosis of each specimen was made by two experienced pathologists. Among them, 3365 patients’ EGFR mutation types were tested by ARMS-PCR with an average age of 63.3 ± 10.7 years, 802 NSCLC patients were tested by NGS with a mean age of 63.5 ± 11.1 years. Patients living in Southwest of China for a long time were chosen in this research.
Sample collection, DNA extraction and mutation screening

Tissue samples include surgical resection, bronchoscope biopsy, metastatic biopsy, blood, sputum, cytology of pleural effusion. The cancer samples were obtained from the respective Clinical Departments. The oncologists required EGFR mutation testing based on the individual clinical situations of each patient in order to guide treatment. Samples were analyzed by the Clinical Molecular Medicine Testing Center, the First Affiliated Hospital of Chongqing Medical University to determine their EGFR mutation status. The database about the patients’ age, gender, sample types and mutation types was established. NSCLC were classified according to the 2015 WHO classification\textsuperscript{11}.

**ARMS-PCR**: DNA was aspirated from formalin-fixed paraffin-embedded (FFPE) afterwards using TRIzol® reagent (cat. no. 15596026; Invitrogen; Thermo Fisher Scientific, Inc.), according to the manufacturer’s protocols. DNA concentrations of all samples were determined using a NanoDrop ND-1000 spectrophotometer at 280 nm (Thermo Fisher Scientific, Inc.). The gene mutation of such samples were detected via amplification refractory mutation system polymerase chain reaction (ARMS-PCR) and the thermocycling conditions of PCR were as follows: 1 cycle of 95\degree C for 5 mins; followed by 15 cycles of 95\degree C for 25 sec, 64\degree C for 20 sec, 72\degree C for 20 sec; and then finally 31 cycles of 93\degree C for 25 sec, 60\degree C for 35 sec, 72\degree C for 20 sec. The ARMS-PCR reagents were provided by AmoyDx Diagnostics Co., Ltd. (cat. no. 20143402001).

**NGS (Capture-based targeted DNA sequencing)**

The genomic DNA profiles of tumor tissue samples were performed using capture-based targeted sequencing. The DNA samples were analyzed with the Qubit dsDNA assay (Thermo Fisher Scientific, Waltham, MA). The library was constructed using 68 gene panel (Burning Rock Biotech Ltd., RS0323F-V2, Guangzhou, China). DNA was fragmented by Covaris M220. Then, repair the end, phosphorylation and adaptor ligation were performed. DNA fragments of 200–400 bp in size were selected by Agencourt AMPure beads (Beckman Coulter, Brea, CA, USA), following by hybridization with capture probes baits. Then, hybridization selection with magnetic beads and polymerase chain reaction (PCR) amplification were performed. The quality and size of the fragments were analyzed. 50 ng of DNA was used for library construction. Twelve PCR cycles were used for library amplification. Then, the indexed samples were sequenced on a miniseq sequencer (Illumina, San Diego, CA) with paired-end reads, and the Miniseq High Output Reagent Cartridge reagent was used. The entire exon regions of EGFR were detected, including single nucleotide variation (SNV) within +/- 20bp, short insertion or deletion variation (INDEL), gene copy number variation (CNV), and breakpoints occur within the capture range gene rearrangement.

**Statistical analyses**

Patients were classified as mutated or wild-type based on the presence of EGFR mutations. The quality and quantity of the material was measured by the total number or percentage of neoplastic cells to determine whether the sample could be analyzed. Their association with the clinical data was tested using Fisher's
Results

Detection of EGFR Gene Mutation in 3665 Patients with NSCLC by ARMS-PCR

Among 3665 NSCLC patients (2042 male and 1623 female) using ARMS-PCR, 1744 cases had EGFR gene mutation, and the total mutation rate was 47.6%. Among 2042 NSCLC male patients, 679 cases had EGFR gene mutations, and the mutation rate was 33.3%. 1065 out of 1623 NSCLC female patients were identified to have EGFR activating mutations, with the mutation rate of 65.6%. Among 3665 NSCLC patients, there was statistically significant (P=0) difference between the female and male cases in the mutation rates of EGFR. And patients, whose age ranged from 45 to 59 years old, had the highest EGFR gene mutation rate among patients of all ages, and the gene mutation rate was 50.9% (611/1200), with statistically significant difference among other different age groups (P=0.005) (There was no statistical significance because patients over 90 years old had fewer cases and a large margin of error.) Sample types of all cases included biopsies of diseased tissues, metastatic tissues, cytological examinations, sputum fluids, whole blood and the mutation rates of EGFR gene were respectively 48.7% (1032/2118), 48.5% (126/260), 48.4% (522/1078), 28.8% (40/139) and 34.3% (24/70), also with statistically significant difference between different sample types (P=0). The characteristics of patients and EGFR mutation rate were shown in Table 1.

Table 1

NCSLC patients EGFR mutation rate and clinical information analysis by ARMS-PCR
## Detection of EGFR Gene Mutation in 296 Patients with Advanced NSCLC by NGS

Totally, 802 NSCLC patients (468 male and 334 female) were further detected by NGS. Among them, 340 cases had EGFR gene mutation, and the total mutation rate was 42.2% (340/802). There were 141 male and 199 female in the EGFR gene mutation cases, and the gene mutation rate was 30.1% (141/468) and 59.6% (199/334) respectively which had statistically significant (P=0) difference. And patients, whose age ranged from 45 to 59 years old, had the highest EGFR gene mutation rate among patients of different ages, and the gene mutation rate was 48% (123/256), without statistically significant difference among different age groups (P=0.13). Sample types of all cases contained biopsies of diseased tissues, metastatic tissues, cytological examinations, sputum fluids, whole blood and the mutation rates of EGFR gene were respectively 46.6%(206/442), 50%(1/2), 63.3%(31/49), 50%(4/8), 32.6%(98/301). And the difference was statistically significant among different sample types(P=0). All characteristics of patients and EGFR mutation rate were shown in Table 2.
Table 2

NCSLC patients EGFR mutation rate and clinical information analysis by NGS

| Overall patients | Case(n) | EGFR Positive | Mutated type | Wild type | χ² | P |
|------------------|---------|---------------|--------------|-----------|----|---|
|                  |         |               |              |           |    |   |
| Sex              |         |               |              |           |    |   |
| Male             | 468     | 141           | 327          | 30.1%     | 69.23 | 0.00 |
| Female           | 334     | 199           | 135          | 59.6%     |      |    |
| Age              |         |               |              |           |    |   |
| <45              | 32      | 12            | 20           | 37.5%     | 12.087 | 0.13 |
| 45~59            | 256     | 123           | 133          | 48.0%     |      |    |
| 60~74            | 379     | 163           | 216          | 43.0%     |      |    |
| 75~89            | 131     | 42            | 89           | 32.1%     |      |    |
| >90              | 4       | 0             | 4            | 0.0%      |      |    |
| Sample type      |         |               |              |           |    |   |
| Tumor Tissue     | 442     | 206           | 236          | 46.6%     | 24.109 | 0.00 |
| Metastatic Tissue| 2       | 1             | 2            | 50.0%     |      |    |
| Cytology         | 49      | 31            | 18           | 63.3%     |      |    |
| Sputum           | 8       | 4             | 4            | 50.0%     |      |    |
| Blood            | 301     | 98            | 202          | 32.6%     |      |    |

**EGFR Gene Mutation unit types detected by ARMS-PCR in 3665 Advanced NSCLC patients**

3665 NSCLC patients were detected EGFR Gene Mutation unit types using ARMS-PCR, 1744 cases had mutation units, which including single site mutation in 1654 patients and multiple locus mutation in 90 patients. Total mutation rate was 47.6%. The total number of positive EGFR mutation units was 1838, due to multiple mutation. The total mutation rates of exon 18, 19, 20 and 21 were 1.7%, 20.7%, 3.1% and 24.7% respectively, and their composition ratios were 3.4%, 41.2%, 6.2% and 49.3%. Among seven mutation types, unit points mutation rate was G719X (1.7%), 19 del (20.7%), S768I (0.7%), T790M (1.1%), 20ins (1.3%), L858R (23.4%), L861Q (1.3%) which has been presented in table 3. Among these NSCLC patients using ARMS-PCR, exon 19 deletion and exon 21 mutations were the main EGFR mutation types which accounted...
for 90.5% of total mutation cases. It is of great significance for guiding targeting therapy with TKI in clinic to those patients with advanced NSCLC.

Table 3

Characteristics of mutation units types of EGFR in 3665 NSCLC patients analysis by ARMS-PCR
| Exons | Sites | Mutations | Amino acid sequence changes | Unit positive Case (n) | Unit Point mutation rate (%) | Total mutation rate of exon (%) | Composition ratio (%) |
|-------|-------|-----------|-----------------------------|------------------------|-----------------------------|--------------------------------|---------------------|
| 18    | 3     | G719X     |                             | 62                     | 1.7%                        | 1.7%                           | 3.4%                |
|       |       | G719A     | 2156G>C                     |                        |                             |                                |                     |
|       |       | G719S     | 2155G>A                     |                        |                             |                                |                     |
|       |       | G719C     | 2155G>T                     |                        |                             |                                |                     |
| 19    | 19    | 19del-    |                             | 757                    | 20.7%                       | 20.7%                          | 41.2%               |
|       |       | E746-A750del | 2236-2250del             |                        |                             |                                |                     |
|       |       | S752-I759del | 2254-2277del             |                        |                             |                                |                     |
|       |       | The rest are not listed |                   |                        |                             |                                |                     |
| 20    | 5     | T790M     | 2369C>T                     | 39                     | 1.1%                        | 3.1%                           | 6.2%                |
|       |       | S768I     | 2303G>T                     | 26                     | 0.7%                        |                                |                     |
|       |       | 20Ins     |                             | 49                     | 1.3%                        |                                |                     |
|       |       | H773-V774insH | 2319-2320insCAC            |                        |                             |                                |                     |
|       |       | D770-N771insG | 2310-2311insGGT            |                        |                             |                                |                     |
|       |       | V769-D770insASV | 2307-2308insgccagctg       |                        |                             |                                |                     |
| 21    | 2     | L858R     | 2573T>G                     | 858                    | 23.4%                       | 24.7%                          | 49.3%               |
|       |       | L861Q     | 2582T>A                     | 47                     | 1.3%                        |                                |                     |

NSCLC: non-small cell lung cancer; EGFR: epidermal growth factor receptor; §: include 1654 single site mutation patients and 90 multiple locus mutation patients

Note: The mutation of EGFR gene in patients with advanced NSCLC was detected by ARMS-PCR. Unit point mutation rate=the number of mutations per unit point/total number of mutations; total mutation rate of exons=the number of exon mutations/total number of mutations; composition ratio=the number of mutations/ total number of mutations

**EGFR Gene Mutation unit types detected by NGS in 802 Advanced NSCLC patients**
A total 802 advanced NSCLC patients were further detected for EGFR Gene Mutation units types by NGS. Among them, there were 209 single site mutation patients and 131 multiple locus mutation patients. The total mutation rate was 42.4%, and the single site and multiple locus mutation rate were 26.1% and 16.3% respectively. The total mutation rates of exon 18, 19, 20, 21 and gene amplification were 1.9%, 15.5%, 6.9%, 20.9% and 7.2% respectively, and their composition ratios were 3.5%, 29.2%, 13.0%, 49.3% and 13.7%. Among them, unit point mutation rate was G719X (1.4%), E709 (0.5%), 19del (15.5%), S768I (0.5%), T790M (3.9%), 20Ins (2.0%), L858R (19.5%), L861Q (1.4%) which has been presented in table 4. Otherwise, there were 5 rare mutations detected including p.R547*, p.R677C, p.E1079K, p.C624Y and EGFR-PPP1R17. In patients with advanced NSCLC detected by NGS, exon 19 deletion and exon 21 mutation were still the main types of EGFR gene mutations, while mutation rates of exon 19 and 21 detected by NGS were significantly lower than which detected by ARMS-PCR. However, the detection of multiple locus mutation was much higher than that of ARMS-PCR which had important clinical significance for better selection of targeted diagnosis and therapy in advanced NSCLC.

Table 4

Characteristics of mutation units types of EGFR in 802 NSCLC patients analysis by NGS
| Exons | Sites | Mutations | Amino acid sequence changes | Unit positive Case (n) | Unit Point mutation rate (%) | Total mutation rate of exon (%) | Composition ratio (%) |
|-------|-------|-----------|----------------------------|------------------------|-------------------------------|---------------------------------|----------------------|
| 18    | 3     | G719X     |                            | 11                     | 1.4%                          | 1.9%                            | 3.5%                 |
|       |       | G719A     | 2156G>C                    |                        |                               |                                 |                      |
|       |       | G719S     | 2155G>A                    | 4                      | 0.5%                          |                                 |                      |
|       |       | G719C     | 2155G>T                    |                        |                               |                                 |                      |
|       |       | E709      |                            |                        |                               |                                 |                      |
| 19    | 19    | 19del-    |                            | 124                    | 15.5%                         | 15.5%                           | 29.2%                |
|       |       | E746-A750del | 2236-2250del               |                        |                               |                                 |                      |
|       |       | S752-I759del | 2254-2277del               |                        |                               |                                 |                      |
|       |       | The rest are not listed | |                        |                               |                                 |                      |
| 20    | 5     | T790M     | 2369C>T                    | 31                     | 3.9%                          | 6.9%                            | 13.0%                |
|       |       | S768I     | 2303G>T                    | 4                      | 0.5%                          |                                 |                      |
|       |       | 20Ins     |                            | 16                     | 2.0%                          |                                 |                      |
|       |       | The rest are not listed | |                        |                               |                                 |                      |
| 21    | 2     | L858R     | 2573T>G                    | 156                    | 19.5%                         | 20.9%                           | 49.1%                |
|       |       | L861Q     | 2582T>A                    | 11                     | 1.4%                          |                                 |                      |
| Rare mutation | 5     |           |                            | 5                      | 0.6%                          |                                 | 1.2%                 |
| 5     |       | p.R547*   |                            |                        |                               |                                 |                      |
| 17    |       | p.R677C   |                            |                        |                               |                                 |                      |
| 27    |       | p.E1079K  |                            |                        |                               |                                 |                      |
| 15    |       | p.C624Y   |                            |                        |                               |                                 |                      |
| Exon28_Intron4 |       | EGFR-PPP1R17 |                          |                        |                               |                                 |                      |
| Gene amplification | |           |                            | 58                     | 7.2%                          | 13.7%                           |                      |

NSCLC: non-small cell lung cancer; EGFR: epidermal growth factor receptor; §: include 209 single site mutation patients and 131 multiple locus mutation patients
Note: The mutation of EGFR gene in patients with advanced NSCLC was detected by ARMS-PCR. Unit point mutation rate=the number of mutations per unit point/total number of mutations

Comparison and analysis of the two methods for detecting mutation rates of EGFR gene

By detecting NSCLC patients with two different ways, ARMS-PCR and NGS, we had compared and analysed the mutation rates of the two methods. Surprisingly, the result had showed that the difference between the EGFR mutation rate in patients determined by ARMS-PCR and those by NGS was significant. Although ARMS-PCR and NGS showed exon 19 deletion and exon 21 mutation were both the main mutation types, the difference of mutation rates was statistically significant. Then we compared the mutation rates of exon 18, 19, 20, 21 and multiple locus mutations by row X list chi-square test. The results showed that the total mutation rates detected by ARMS-PCR were higher than those by NGS (47.6% vs. 42.4%, P<0.05), however, the multiple locus mutation rate detected by ARMS-PCR was much lower than those by NGS (2.7% vs. 16.3%, P<0.001) (table 5). Except for the difference in exon 18 mutation rate which was similar between the two methods, the mutation rate in exon 19, 20 and 21 had statistical difference. This results suggested that ARMS-PCR had higher detection rate and higher sensitivity than NGS in single site gene mutation of EGFR, which could meet the needs of clinicians to select the EGFR-TKI targeted drugs quickly and accurately. In contrast, NGS had significant advantages in detecting multiple and rare mutation, which could meet the needs of personalized medicine better.

Table 5

Comparison of the two methods for detecting mutation rates of EGFR gene
### Discussion

At present, lung cancer remains one of the most common cancers in China. The mortality and morbidity of lung cancer is still one of the fastest-growing cancers in recent years in China\(^1\). NSCLC accounts for approximately 85% of lung cancer cases, which seriously threatens human health and life\(^1\). EGFR-TKI is a representative drug, the efficacy of which has been clinically confirmed. However, the sensitivity and prognosis of EGFR-TKI treatment is associated with the status of EGFR gene mutation, and the treatment has a significant impact on sensitive mutant patients, while the prognosis of non-mutant patients is poor\(^1\).\(^4\).\(^5\). So, in order to promote the therapeutic effect, the EGFR gene mutation status is supposed to be cleared before carrying out the clinical therapy of NSCLC patients\(^6\). There are differences in EGFR gene mutations in NSCLC in different regions and different races\(^7\).

In this study, the clinical data of 4467 patients with NSCLC had been collected in Southwest of China, and the total mutation rate of EGFR gene was 46.7%, similar to the 48.7% EGFR mutation rate reported by the West China Hospital of Sichuan University in China. In our research, the most frequent mutation types of

| Relevant factors | Method    | Wild type | Mutant type | Mutation rate | \(\chi^2\) | P    |
|-----------------|-----------|-----------|-------------|---------------|-----------|------|
| Exon 18         | ARMS-PCR  | 3603      | 62          | 1.8%          | 0.124     | 0.725|
|                 | NGS       | 787       | 15          | 1.9%          |           |      |
| Exon 19         | ARMS-PCR  | 2908      | 757         | 20.7%         | 11.21     | 0.001|
|                 | NGS       | 678       | 124         | 15.5%         |           |      |
| Exon 20         | ARMS-PCR  | 3551      | 114         | 3.1%          | 25.384    | 0.000|
|                 | NGS       | 747       | 55          | 6.9%          |           |      |
| Exon 21         | ARMS-PCR  | 2760      | 905         | 24.7%         | 5.403     | 0.020|
|                 | NGS       | 635       | 167         | 39.4%         |           |      |
| Multiple locus  | ARMS-PCR  | 90        | 90          | 2.7%          | 269.319   | 0.000|
| mutations       | NGS       | 131       | 131         | 16.3%         |           |      |
| Total           | ARMS-PCR  | 1921      | 1744        | 47.6%         | 7.125     | 0.008|
|                 | NGS       | 462       | 340         | 42.4%         |           |      |

Note: Comparison of mutation rates of exon-related mutation sites detected by the two methods. The mutation rates were compared by row X listchi-square test, and P.

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\(^1\) \(12\) \(13\) \(14\) \(15\) \(16\) \(17\)
EGFR were exon 19 deletion and exon 21 mutation (L858R), which resembled those that reported in researches performed in the countries of East Asia \(^{18-21}\). According to previous studies, the EGFR gene mutations in NSCLC patients are related to race, sex, smoking history, pathological type, and sample type \(^5,19,22\). And this research further confirms that EGFR gene mutations are related to sex and sample type. Therefore, both previous studies and the current study have shown that EGFR mutations are more common to female, never-smoker and lung adenocarcinoma, and this group of people is more likely to benefit from EGFR-TKI drugs, which could help to choose the best therapeutic drugs for clinicians with a significant reference.

At present, ARMS-PCR and NGS are the main methods in EGFR gene mutation detection. The application of NGS is increasing as a kind of new technology which is being used to test inherited disorders and different tumor gene mutations \(^{23}\). NGS has great significance for the further step of personalized medicine by detecting the somatic driver mutations, mutational burden quantification, resistance mechanisms, germline mutations, which also has the ability to sequence circulating tumor DNA (ctDNA) in liquid biopsy for screening and early diagnosis \(^{24-25}\). In many cases of lung cancer, NGS could avoid the difficulties by detecting ctDNA, as the biopsy is a painful procedure for patients \(^{26}\). Many reports have indicated that the advantages of NGS was high sensitivity in detecting actionable alterations used by gene panel \(^{27-29}\). Especially in Asian predominant population, NGS represents a non-invasive, cost-effective way of diagnosis compared with sequential testing strategies \(^{30}\). However, challenge follows with promise. For example, the increasing generation of enormous sequence presents a huge challenge for data integration, analysis and interpretation \(^{31}\). NGS could easily discover numerous genetic variations between tumor and normal tissue, but it will be hard to extract clinically useful and actionable information and validate the significant genotype-phenotype associations \(^{32}\). The related bioinformatics challenge such as CNVs, SNVs and epigenetic variations all pose difficulties in detection and are therefore associated with higher error rates \(^{33,34}\).

In clinical practice, NGS also meets the problem of high cost in time and economy. From nuclein extraction to report diagnosis, it will take up 3 ~ 5 days and the high expense of machine and reagent make it hard to popularize in the primary hospitals in China.

ARMS-PCR has been widely used since the end of 2015 to replace the traditional Sanger sequencing in domestic hospital to detect the mutation type of EGFR gene which is based on quantitative polymerase chain reaction (qPCR) \(^{35}\). ARMS-PCR has been approved by the China Food and Drug Administration (CFDA) which is sensitive and reliable for detecting EGFR gene mutation \(^{36}\). The advantages of this method are mature, simple, reliable, and the experimental operation is easy, the detection period is short, which could avoid the toxic operation and detect early genetic alteration that could better meet the current clinical needs \(^{37}\). The reason for ARMS-PCR with the high sensitivity is its peculiar primer design, one pair of primers could amplify a conserved region, and another primer pair could target the point mutation. ARMS-PCR applies only in the detection of known mutations, each reaction system could only detect the pre-specified gene mutation. Therefore, a large amount of DNA samples and primer pairs are needed, if an unknown gene mutation region must be detected and analyzed, which will make the detection method expensive such as NGS. Although the ARMS-PCR is the main mean to detect EGFR gene mutations \(^{36}\), it is considered as an
alternative method because the NGS has the advantages of high flux and diverse detection types in detecting gene mutations\textsuperscript{10}.

Learning from our data, the proportion of EGFR mutations detected by such two methods was basically consistent with previous reports, and the main mutations were exons 19 and 21. But there was some statistically significant difference in the composition ratio of exon 19 mutation between NGS and ARMS-PCR with 29.2\% vs 41.2\% (P < 0.05). About mutation rate, the total mutation rate of EGFR gene detected by ARMS-PCR was 47.6\% which was higher than that detected by NGS (42.4\%). The single mutation rates detected by ARMS-PCR in exons 18, 19, 20 and 21 were 1.8 \%, 20.7\%, 3.1\% and 24.7\%, respectively, and the single mutation rates detected by NGS in exons 18, 19, 20 and 21 were 1.9 \%, 15.5\%, 6.9\% and 39.4\%. The mutation rate detected by NGS was much higher than that by ARMS-PCR in exons 20 and 21, however, the mutation rate detected by NGS was lower than that by ARMS-PCR in exon 19. The difference was statistically significant, but there was no significant difference in exon 18. Such difference may be associated with the type of sample, the number of subjects, different instruments and reagents. The T790M and 20-ins were resistant mutation units, which suggested that patients with sensitive mutations developed resistance during medical treatment. Surprisingly, the multiple mutation rate detected by NGS was 16.3\% which was much higher than the 2.7\% detected by ARMS-PCR with statistically different. At present, the standard care for patients that resist TKI drug caused by the EGFR T790M mutation is to use the third-generation TKI drugs which may be important to direct the drug use for the resistant patients by using NGS. However, some rare loci can be detected by NGS but the importance and directed meaning are still unknown, and the number of rare mutations is rare too. Therefore, NGS has already been used to identify new biomarker candidates for the early diagnosis of lung cancer and is increasingly used to guide personalized treatment decisions\textsuperscript{38} and could be used to explore the mechanism of drug resistance.

Conclusions

Further research is still needed to direct drug use and assess therapy prognosis. With the increasing of drug resistance to the therapy, the EGFR gene mutation should be monitored in patients with poor therapeutic effects when conditions permit. In targeted treatment of lung cancer, NGS has a higher throughput and a wide range of detections than ARMS-PCR technology, and when there are fewer clinical biopsy samples, more information on genetic mutations can be obtained at once, providing a more comprehensive therapeutic basis for the clinic.

Abbreviations

NSCLC, non-small cell lung cancer; ARMS-PCR, qPCR, quantitative polymerase chain reaction; amplification refractory mutation system PCR; NGS, next generation sequencing; EGFR, epidermal growth factor receptor; ctDNA, circulating tumor DNA; CFDA, China Food and Drug Administration.

Declarations

Ethics approval and consent to participate.
This study was approved by the Ethics Committee of the First Affiliated Hospital of Chongqing Medical University Ethics Review Board.

**Consent for publication.**

All authors consent for publication and all patients provided written patient consent for publication.

**Availability of data and material.**

All data generated or analyzed during this study are included in this published article.

**Competing interests.**

None.

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**Authors' contributions.**

CW, JW, SC, YW, YS and HB carried out the sample collection, laboratory detection and drafted the manuscript. LC and XL participated in the design of the study and performed the statistical analysis. ZY, XC and XL conceived of the study, and participated in its design and coordination and helped to draft the manuscript. All authors read and approved the final manuscript.

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