Next generation sequencing based detection of 15 target genes mutations in papillary thyroid carcinoma

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Abstract

Objectives: Next generation sequencing (NGS) method provided a valuable tool for a comprehensive understanding of papillary thyroid carcinoma (PTC) biology. We explored the application of NGS based detection for a panel of 15 target genes mutations and analyzed the gene spectrum in PTC.

Methods: A total of 211 formalin-fixed, paraffin-embedded (FFPE) tissue specimens from surgically removed PTC samples were collected and detected with 15 target genes by NGS.

Results: In general, 181 mutations of all types of 15 target genes were detected in 164 samples (77.73% of 211 samples). There were 22.27% samples with no mutation, and 70.14% samples carried mutations in single gene. A total of 7.11% samples simultaneously harboured two gene mutations and 0.47% sample carried triple different gene mutations. The BRAF mutation was the most common mutation type, followed by TERT, RET fusion, TP53, PIK3CA, GNAS, NTRK1 fusion, CTNNB1, NRAS, and HRAS. TSHR, AKT1, PETN, KRAS, and PAX8 mutations were negative among PTC patients. All point mutations found in PTC samples were heterozygous mutations with allelic frequency ranged from 1.12% to 48.04% of alleles. The fusion mutations showed the percent of reads ranged from 2.30% to 55.54%. For samples with carried two mutations, the allelic frequency of mutations was similar. However, for the sample harboured triple gene mutations, the allelic frequency of NRAS mutation was similar to TERT mutation (33.62%-39.67%) and TP53 mutation showed much lower abundance (4.13%).

Conclusions: NGS-based detection method showed advantages in detecting multiple gene mutations simultaneously, economically and efficiently, and providing quantitative assessment of mutation frequency using targeted sequencing panel. It can improve the accuracy of diagnosis and prognostication and is useful for providing personalized treatments for PTC patients.

Keywords
gene spectrum, mutations, next generation sequencing, papillary thyroid carcinoma

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INTRODUCTION

Thyroid cancer (TC) is the most common endocrine tumor, and the incidence of TC has increased over the past years.1-3 Papillary thyroid carcinoma (PTC) is the most common subtype of TC, represents approximately 80% to 90% of all TCs.4

In the past, single gene assays, such as BRAF V600E mutation, have been commonly used for finding molecular alterations by Sanger sequence, immunohistochemistry, and real time PCR in PTC.5,6 Recently, published literature increasingly suggested that somatic mutations in BRAF, HRAS, KRA S, NRAS, TERT, RET, PIK3CA, PTEN, TP53, CTNNB1, AKT1, GNAS, PAX8/PPARγ, NTRK1, and TSHR genes have been associated with diagnosis and treatment of TC.7-16 Next generation sequencing (NGS) is a newly-developed technique offered a cost-effective approach for detecting multiple genetic alterations and provided quantitative assessment of mutation frequency.17,18 Therefore, a panel designed to these 15 target genes was used to detect molecular alterations by NGS in PTC.

In this study, we focused on the application of NGS based detection of 15 target gene mutations and analyzed the mutation profiles in PTC.

MATERIALS AND METHODS

2.1 | PTC samples

A total of 211 formalin-fixed, paraffin-embedded (FFPE) tissue specimens from surgically removed PTC samples were collected in Jiangsu Cancer Hospital between November 2018 and May 2020. Based on histological results, all tumors were classified by two independent pathologists.

2.2 | DNA extraction

DNA extraction used the QIAamp FFPE Tissue Kit (Qiagen, Hilden, Germany). DNA concentration was measured using a Qubit assay.

2.3 | NGS library preparation

DNA was sheared to short fragments using huber Minichiller 300 (Huber, Offenburg, Germany). DNA fragments were end repaired, phosphorylation and adaptor ligation. The capture beads (Beckman Coulter, Brea, California) were then used to select the target DNA fragments. Afterward, DNA PCR amplification was performed. The resulting mixture was purified, followed by hybridization with capture probe baits and selection with beads. The mixtures were then amplified. The final indexed libraries were pooled and sequenced on the Novaseq 6000 instrument (Illumina, San Diego, California).

2.4 | Capture-based target enrichment

A panel consisting of critical exons and introns of BRAF, NRAS, KRAS, HRAS, TERT, RET, PIK3CA, PTEN, TP53, AKT1, GNAS, CTNNB1, PAX8/PPARγ, TSHR, and NTRK genes (RIGEN, Shanghai, China) was used.

2.5 | Bioinformatics

The raw data were mapped to the human genome (hg19) by BWA Aligner 0.7.10, filtered through GATK 4.0.2.0 and VarScan.v2.3.9. Fusion mutation analysis was performed with Factera 1.4.4. Mutations present in at least 1% of the total number of reads were considered for mutational calls.

RESULTS

3.1 | Clinical characteristics

A total of 211 PTC samples were analyzed. Of the included patients, 71(33.65%) were male and 140(66.35%) were female, and the age ranged from 11 to 75 years (>55 years, 41(19.43%) and <55 years, 170(80.57%).

3.2 | Gene spectrum in PTC

In general, 181 mutations of all types of 15 target genes were detected in 164 samples (77.73% of 211 samples). There were 22.27% samples (47 of 211 samples) with no mutation in 15 target genes. And 70.14% samples (148 of 211 samples) carried mutations in single gene. A total of 7.11% samples (15 of 211 samples)

FIGURE 1

Proportion of patients carrying different gene mutations
simultaneously harboured two gene mutations and 0.47% sample (1 of 211 samples) carried triple different gene mutations (Figure 1). The BRAF mutation was the most common mutation type, followed by TERT, RET fusion, TP53, PIK3CA, GNAS, NTRK1 fusion, CTNNB1, NRAS, and HRAS. TSHR, AKT1, PETN, KRAS, and PAX8 mutations were negative among PTC patients (Figure 2).

The BRAF mutations were all typical p.V600E mutations. Among the RET fusion mutations, 10 were CCDC6/RET fusion, 1 were NCOA4/RET fusion and 1 were SPECC1L/RET fusion. Among the TERT mutations, 11 were C228T and 1 was C250T.

Fifteen PTC patients had two gene mutations simultaneously, including BRAF/TERT (N = 11), BRAF/CTNNB1 (N = 1), BRAF/GNAS (N = 1), BRAF/TP53 (N = 1), and BRAF/PIK3CA (N = 1). 1 PTC patients harboured TERT, NRAS, and TP53 mutations simultaneously.

3.3 | Mutation allele frequency

In the present study, all point mutations found in PTC samples were heterozygous mutations with allelic frequency ranged from 1.12% to 48.04% of alleles (which corresponds to 2.24% to 96.08% of cells with a heterozygous mutation). The fusion mutations showed the percent of reads ranged from 2.30% to 55.54%. For samples with carried two mutations, the allelic frequency of mutations was similar. Interestingly, for the sample harboured triple gene mutations, the allelic frequency of NRAS mutation was similar to TERT mutation (33.62%-39.67%) and TP53 mutation showed much lower abundance (4.13%).

4 | DISCUSSION

A complete understanding of the molecular mechanisms of tumor formation is essential for providing accurate diagnoses and personalized treatments. In this study, NGS-based detection method was used for detecting 15 target gene mutations, which offered a valuable tool for a comprehensive understanding of PTC biology.

Due to sample size limitations and patients from specific geographic locations, some mutations such as TSHR, AKT1, PETN, KRAS, and PAX8 mutations were negative in the present study, which varied from previous studies.7,10,19,20 PTC samples were dominated by BRAF V600E mutations which were consistent with the previous studies showed the BRAF V600E mutation rate was 29% to 90%.4,16,20-22

Ke et al. showed the mutation types were point mutations, indel (insert and deletion) mutations and gene fusions in PTC.20 However, only point mutations and gene fusions were detected in current study.

A recent study reported TP53 and PTEN mutations showed allelic frequency of more than 50%, suggesting either amplification of the mutant allele in tumor cells or a loss of the wild-type allele.23 However, in the present study, no point mutations showed allelic frequency of more than 50%. Two CCDC6/RET fusion mutations showed the percent of reads more than 50%. For samples with carried two mutations, the allelic frequency of mutations was similar, indicating that these mutations were in the same clonal population of cells. Interestingly, for sample harboured TERT, NRAS, and TP53 mutations simultaneously, the allelic frequency of TP53 mutation showed much lower than the others. This finding may be due to TP53 mutation was late events in tumor clone progression.

In conclusion, NGS-based detection method showed advantages in detecting multiple gene mutations simultaneously, economically and efficiently, and providing quantitative assessment of mutation frequency using targeted sequencing panel. It can improve the accuracy of diagnosis and prognostication and is useful for providing personalized treatments for PTC patients.

CONFLICT OF INTEREST

The authors declare that we have no competing interest.

AUTHOR CONTRIBUTIONS

Zhuo Wang: conception, design and manuscript writing
Changwen Jing: collection and assembly of data
Haixia Cao: data analysis
Siwen Liu: data analysis
Jianzhong Wu: conception and design
Rong Ma: conception and design
All authors: final approval of manuscript.

ETHICS STATEMENT

The study was approved by Nanjing Medical University and all patients gave written informed consent.

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FIGURE 2 Target gene mutations in papillary thyroid carcinoma.
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