Cellular and Molecular Biology
E-ISSN : 1165-158X / P-ISSN : 0145-5680
www.cellmolbiol.org

Detection of TP53 gene mutation in blood of breast cancer patients based on circulating tumor DNA and its application in prognosis

Qiran Lu, Luxin Wei, Shenbin Cai, Zhe Zhang, Lifeng Zhang*

Department of General Surgery, Dushu Lake Hospital Affiliated to Soochow University, Suzhou, China

ARTICLE INFO

Original paper

Article history:
Received: June 25, 2023
Accepted: September 22, 2023
Published: November 15, 2023

Keywords:

Breast cancer, TP53, gene mutations, CtDNA, prognosis

ABSTRACT

This experiment was carried out to explore the application value of high throughput gene sequencing technology in detecting TP53 gene mutations in the blood of patients with breast cancer by detecting ctDNA gene mutations, and exploring the relationship between TP53 mutations and clinicopathological characteristics and prognosis of patients. The gene mutation of peripheral blood ctDNA and tissue paraffin DNA (tDNA) of 50 patients was detected by high-throughput sequencing technology. The basic data of 50 cases of Medium to high-risk breast cancer diagnosed and were retrospectively collected, and the clinicopathological characteristics and survival results of TP53 mutant and wild-type patients were compared and analyzed according to the ctDNA detection results and relevant follow-up data. Analyze the impact of TP53 mutations on overall survival and progression-free survival using univariate and multivariate Cox regression models. Among 50 patients, there were 29 cases of 7 kinds of gene mutations detected by ctDNA, and 37 cases of 9 kinds of gene mutations detected by tDNA. Using the gene mutation results detected by tDNA as the gold standard, the sensitivity and specificity of peripheral blood ctDNA in diagnosing TP53 gene mutations are 75% to 100%, 92.31% to 100%, and the overall coincidence rate with tDNA results was 83.33% to 100%. Exon 5 was the most prone to mutation, with a frequency of 24.14% (7/29). The most common type of mutation was the missense mutation of 37.93% (11/29). There was no significant correlation between TP53 mutation and PFS (HR=0.67, 95% CI: 0.41-1.08, *P*=0.102), while TP53 mutation was a protective factor for OS (HR=0.49, 95% CI: 0.27-0.90, *P*=0.022). The detection of ctDNA in peripheral blood of breast cancer patients by high-throughput gene sequencing technology can replace tumor tissue sections to understand gene mutation. The TP53 mutation in breast cancer patients is related to tumor size, lymph node metastasis and vascular tumor thrombus, but the prognosis of TP53 mutant patients is similar to that of wild-type patients.

Doi: http://dx.doi.org/10.14715/cmb/2023.69.11.30

Introduction

Breast cancer is the cancer with the highest incidence rate among women and one of the tumors with a high Case fatality rate. In 2023, it is estimated that there will be 231840 newly confirmed cases in the United States. Except for lung cancer, breast cancer has more deaths than any other part of the cancer (1). In China, the incidence rate of breast cancer is on the rise year by year, becoming the main cancer burden for Chinese women. Before the 1990s, breast cancer was not common in China, but now more than 1.6 million people are diagnosed with cancer every year and 1.2 million people die of cancer (2). Like most other countries, breast cancer is now the most common cancer among Women in China; China's breast cancer cases account for 12.2% of newly diagnosed breast cancer cases and 9.6% of global breast cancer deaths (3). It has become one of the main causes of death for cancer patients in China. Accurately obtaining the biological information of tumors for personalized treatment under the guidance of genotype is crucial for guiding clinical medication. At present, tissue DNA (tDNA) is commonly used in clinical practice for genotyping, but this detection is invasive and requires high requirements for specimen acquisition. Circulating tumor DNA (ctDNA) refers to small pieces of DNA released into the circulatory system after tumor cell Somatic cell DNA falls off or cell apoptosis. ctDNA comes from Somatic cell mutations of tumor cells, which can have the same characteristics or gene changes as the primary tumor DNA. Peripheral blood ctDNA can be qualitatively, quantitatively, and tracked, which may provide a series of convenient, fast, specific, non-invasive or minimally invasive molecular biological detection methods for early diagnosis, prognosis determination, and follow-up of clinical tumors. However, it is unknown whether ctDNA in peripheral blood can replace tDNA as a routine Genetic testing method for clinical application.

TP53 (Tumor suppressor P53) is a tumor suppressor gene, located in the short arm of chromosome 17. Because of its functions in cell cycle regulation, cell apoptosis and DNA repair, it is called the "guardian of the genome" (4), and its coding product is the transcription factor P53 protein. P53 protein is composed of a transcriptional activation domain, DNA binding domain and multifunctional domain. Mutation of proto-oncogene and DNA damage can directly activate P53. Activation of P53 can induce cell cycle stagnation, and accelerate DNA repair and cell apoptosis, so as to prevent DNA replication errors and cell abnormalities, thus preventing cell malignant transformation (5,6). The mutation of the TP53 gene is considered to be

* Corresponding author. Email: luqiran666666@163.com

an early event of breast cancer, which plays an important role in the occurrence and development of tumors. When the TP53 gene mutates, its encoded P53 protein has a longer half-life, enhanced stability, and can be continuously accumulated in the nucleus. Mutant P53 protein loses the anti-tumor ability of wild-type P53, and obtains functions similar to oncogene expression products, thus enhancing the invasive ability of tumor cells, and promoting the occurrence and development of tumors (7).

TP53 plays an important role in the occurrence and development of breast cancer. The mutation of the TP53 gene can not only promote the growth of cancer but also make patients resistant to treatment. Currently, researchers have been searching for targeted drugs targeting TP53 (8). The Missense mutation of the TP53 gene is the most common mutation in human cancer, and homozygous mutations of the TP53 gene can be detected in 50% -60% of cancers. Previous studies have shown that TP53 mutation in breast cancer is a potential biomarker that leads to poor prognosis and insensitivity to multiple treatment schemes (9).

Therefore, this study used target sequence capture and second-generation sequencing to analyze the TP53 mutation of circulating tumor DNA (ctDNA) in 50 patients with breast cancer. Using tissue tDNA as the gold standard, we evaluated its diagnostic efficacy, as well as the clinicopathological data and follow-up data of breast cancer patients, in order to analyze the clinicopathological characteristics and survival of patients with TP53 Mutant and provide a theoretical basis for the diagnosis and treatment of patients with TP53 Mutant breast cancer.

Materials and Methods

General information

From January 2016 to January 2022, a total of 50 female breast cancer patients with complete initial diagnosis, clinical pathology and follow-up data diagnosed and treated by the medical team were collected, and they met at least one of the following conditions: moderate risk: patients with negative axillary lymph nodes, with lesions>2 cm, pathological grading of 2-3, invasion of tumor peripheral blood vessels, HER-2 gene overexpression or amplification, and any of the age < 35 years old, All patients are at moderate risk, with 1-3 axillary lymph nodes metastasis and no overexpression or amplification of the Her-2 gene. High risk: 1-3 axillary lymph nodes metastasize and the Her-2 gene is overexpressed or amplified, with 4 or more axillary lymph nodes. Exclusion criteria: (i) special pathological type breast cancer; (ii) Received chemotherapy within one month before collecting peripheral blood samples; (iii) Missing data on important clinical variables; (iv) Combined with other tumors or serious diseases; (v) Male. Collect clinical-pathological data of patients for subsequent analysis, mainly including the age of diagnosis, clinical TNM staging, treatment plans (surgery, chemotherapy, radiotherapy, and immunotherapy), and long-term follow-up data.

Gene sequencing

TP53 mutation detection based on peripheral blood ctDNA

The patient's blood is used for ctDNA testing, and the sequencing timing is determined based on clinical needs. Collect peripheral blood samples for ctDNA analysis using Street blood collection vessels (Street, Omaha, NE, USA) and centrifuge (first, 4°C, 1600 × Centrifuge for 10 minutes; Then, transfer to a new micro-centrifuge tube at 4°C, 16000 × Centrifuge for 10 minutes to remove residual cell fragments, and separate plasma from peripheral blood within 72 hours. The target sequence sequenced covers 1.1Mb, including 1021 genes, and covers all Intron and Exon of TP53 (10, 11). Use with 2 × The HiSeq3000 Sequencing System (Illuminata, SanDiego, CA, USA) with 101bp paired-end reading was used for DNA sequencing. Low-quality readings and end joint sequences were eliminated. The reference Human genome (hg19) was calibrated with Burrows-Wheeler Aligner (BWA, version 0.7.12-r1039), and the Genome Analysis Toolkit (GATK, version 3.4-46-gbc02625) was used for re-alignment and calibration. Single nucleotide variants (SNVs) were identified using MuTect (version 1.1.4) and NChot. Use GATK to identify small insertions and deletions, indices.

tDNA extraction and detection

Tumor tissue (paraffin section) was extracted with a Qiagen DNA extraction kit and then broken into 300~350 bp by ultrasound. 96 rxn xGen Exome Research Panel v1 After enrichment with the 0 kit, a high-throughput sequencing library was established, and 600 times deep sequencing was performed using the Illumina Hiseq 4000 high-throughput sequencing platform. The offline data was organized into sample mutation information through the bioinformatics platform.

Treatment and follow-up

Individualized treatment is developed for each patient based on different disease stages, and patients may receive two or more treatment plans. Follow-up was conducted by phone and return to the hospital for follow-up, with follow-up up to June 2023. Among the 50 patients, 58 were followed up, with a success rate of 96.67% and a follow-up period of 1.2-17.6 months. Progression-free survival (PFS) refers to the time from the start of first-line treatment to tumor progression or death from any cause. PFS is calculated based on the visit deadline for patients who have not progressed at the follow-up deadline.

Study endpoint

The basic data of the enrolled patients were collected, and the differences in basic clinical data between the TP53 Mutant and the wild-type patients were compared, and the overall survival and progression-free survival rates of the two groups of patients were compared. Through univariate and multivariate Cox regression analysis, we explored whether TP53 was an independent risk factor affecting the overall survival and progression-free survival of breast cancer patients. Then, we analyzed the common sites and mutation types of TP53 gene mutations in patients with TP53 Mutant.

Statistical Analysis

Chi-squared test and Fisher's exact test were used to compare the Categorical variable. Progress free survival (PFS) is defined as the time from radical mastectomy to recurrence or death from any cause of breast cancer. PFS is defined as the time from the start of treatment to the progression of the disease or death from any cause. Patients

without endpoints (progression or death) were excluded at the last follow-up. Draw Kaplan Meier survival curves based on gene mutation status and compare them using the logarithmic rank test. Use the Cox proportional risk model to analyze the correlation between OS or PFS and gene mutations and clinical features. All statistical tests were bilateral, indicating a statistically significant difference of P<0.05. All statistical analyses were conducted using Statistic Package for Social Science (SPSS) version 23.0 (IBM Corporation, Armonk, NY, USA) or GraphPad Prism 5.0 (GraphPadSoftware, LaJolla, CA, USA).

Results

TP53 mutation results

Among the 50 patients, a total of 21 patients were detected to have TP53 mutations. A total of 29 TP53 mutations were found in 21 patients, of which 2 patients detected 4 mutations, 4 patients detected 3 mutations, and 4 patients detected 2 mutations. As shown in Figure 1, the Exon 5 Exon was most prone to mutation, with a frequency of 24.14% (7/29); The next were Exon 7, 10 and 8. The most common type of mutation was Missense mutation, accounting for 37.93% (11/29), followed by frameshift mutation, accounting for 24.14% (7/29).

Diagnostic efficacy evaluation of peripheral blood ctDNA gene for TP53 gene mutations

Using the TP53 gene mutation results detected by tissue paraffin section tDNA as the gold standard, the sensitivity of peripheral blood ctDNA to TP53 gene mutations is 75% to 100%, the specificity is 92.31% to 100%, and the overall coincidence rate with tDNA results is 83.33% to 100%, as shown in Table 1. Among them, no mutations were detected in the tDNA of 2 patients, but TP53 exon5 deletion and TP53 exon8 mutation were detected in the corresponding peripheral blood ctDNA, respectively (Table 1).

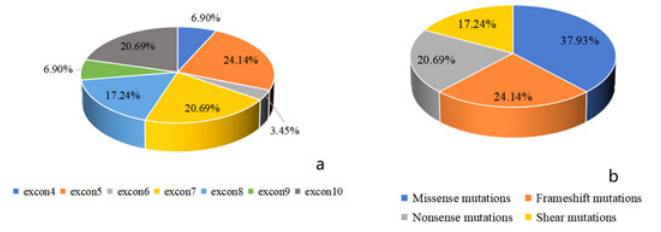

Figure 1. Analysis of mutation sites and types in patients with TP53 mutation; a: Mutation sites; b: Mutation type.

Clinical and pathological characteristics of patients

The paraffin-embedded tissue samples of 50 patients with breast cancer with complete clinical and pathological data were selected, and the samples were reviewed and diagnosed by the disease experts. Immunohistochemical method was used to detect the expression of TP53 in tumor tissues of breast cancer patients, select representative tissue sections and take photos under the microscope, as shown in Figure 2.

A total of 50 patients were included in this study, of whom 27 (54.0%) were over 50 years old; Postmenopausal 26 (52.0%); 43 (86.0%) received neoadjuvant chemotherapy; 24 (48.0%) tumors with a length diameter greater than 2.0cm; 19 (38.0%) had lymph node metastasis; The pathological type is invasive ductal carcinoma 43 (86.0%); Histology Level III 37 (74.0%); Ki-67 > 30% is 37 (74.0%); 32 (64.0%) underwent simple mastectomy. Compared with the wild type, the positive expression rate of ER and PR of TP53 Mutant is higher, and the clinical stage tends to be stage III, with statistically significant differences (P<0.05) (Table 2).

Comparison of transfer rate between patients with TP53 gene Mutant and wild-type patients

After 3 years of follow-up, patients with TP53 gene Mutant had higher rates of bone metastasis, lung metastasis and liver metastasis than those with wild type (*P*<0.05).

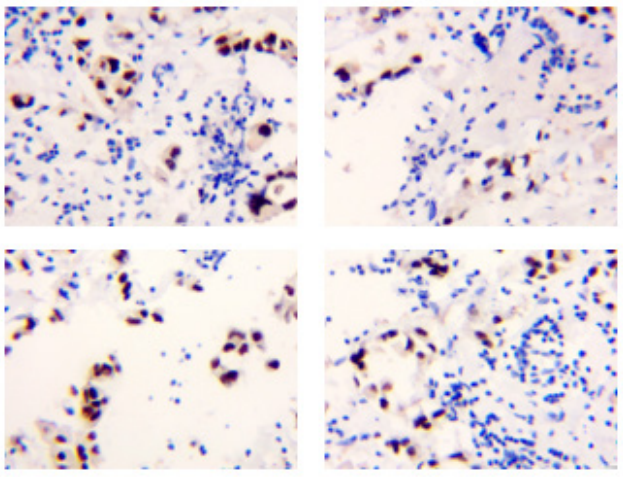

Figure 2. Expression of TP53 in tumor tissue (immunohistochemical staining) × 200).

Table 1. Diagnostic efficacy evaluation of peripheral blood ctDNA for TP53 gene mutations.

TP53 mutation site	ctDNA+(n)		CtDNA - (n)		Sensitivity (%)	Specificity (%)	Overall compliance rate (%)
	tDNA+	tDNA-	tDNA+	tDNA-			
exon3			2	19	-	100	89.47
exon4	2			18	100.00	100	100
exon5	7	1	1	12	87.50	92.31	83.33
exon6	1			20	100.00	100	100
exon7	6		2	13	75.00	100	84.61
exon8	5	1	1	14	83.33	93.34	85.71
exon9	2			19	100.00	100	100
exon10	6		1	14	85.71	100	92.86
exon11			3	18	-	100	83.33

Table 2. Baseline characteristics and distribution of TP53 mutations in patients (n (%)).

Item	Total (n=50)	TP53 mutation (n=31)	TP53 wild type (n=19)	*P*
Age (years)				0.162
≤50	23 (46.0)	14 (45.16)	9 (47.37)	
>50	27 (54.0)	17 (54.84)	10 (52.63)	
Menstrual status				0.417
Premenopausal/	24 (48.0)	11 (35.48)	13 (68.42)	
Postmenopausal	26 (52.0)	20 (64.52)	6 (31.58)	
Neoadjuvant chemotherapy				0.079
Null	43 (86.0)	27 (87.10)	16 (84.21)	
Exist	7 (14.0)	4 (12.90)	3 (15.79)	
Tumor length diameter (cm)				0.491
≤2.0	26 (52.0)	16 (51.61)	10 (52.63)	
>2.0	24 (48.0)	15 (48.39)	9 (47.37)	
Lymph node metastasis				0.739
Positive	31 (62.0)	18 (58.06)	13 (68.42)	
Negative	19 (38.0)	13 (41.94)	6 (31.58)	
Pathological type				0.246
Ductal carcinoma	43 (86.0)	27 (87.10)	16 (84.21)	
Non-ductal carcinoma	7 (14.0)	4 (12.90)	3 (15.79)	
Histological classification				0.067
I/II	13 (26.0)	6 (19.35)	7 (36.84)	
III	37 (74.0)	25 (80.65)	12 (63.16)	
Ki-67 (%)				0.004
≤30	13 (26.0)	5 (16.13)	8 (42.11)	
>30	37 (74.0)	26 (83.87)	11 (57.89)	
Surgical approach				0.209
Breast-conserving	18 (36.0)	10 (32.26)	8 (42.11)	
Simple resection	32 (64.0)	21 (67.74)	11 (57.89)	
Chemotherapy				1
Null	3 (6.0)	2 (6.45)	1 (5.26)	
Exist	47 (94.0)	29 (93.55)	18 (94.4)	
Phantom drugs				0.363
Null	26 (52.0)	16 (51.61)	10 (52.63)	
Exist	24 (48.0)	15 (48.39)	9 (47.37)	
Radiotherapy				0.372
Null	16 (32.0)	11 (35.48)	5 (26.32)	
Exist	34 (68.0)	20 (64.52)	14 (73.68)	
ER				<0.001
Positive	23 (46.0)	16 (51.61)	7 (36.84)	
Negative	27 (54.0)	15 (48.39)	12 (63.16)	
PR				<0.001
Positive	23 (46.0)	18 (58.06)	5 (26.32)	
Negative	27 (54.0)	13 (41.94)	14 (73.68)	

See Figure 3.

Comparison of survival between patients with TP53 gene Mutant and wild-type patients

During 3-year follow-up, the progression-free survival rate and overall survival rate of patients with TP53 gene Mutant were higher than those of wild-type patients, with no statistically significant difference ($P>0.05$), as shown in Figure 4.

Correlation between TP53 mutation and prognosis

A univariate analysis was conducted on the survival prognosis of enrolled patients, and the results showed that age >50 years old, tumor size, presence of lymph node metastasis, and positive vascular cancer thrombus were significantly correlated with PFS (P<0.05, see Table 3). At the same time, the size of the tumor, the presence of lymph node metastasis, and the positivity of vascular cancer thrombi were also significantly correlated with OS (P<0.05, see Table 4). To eliminate the interference of clinical pathological features on the prognosis of TP53, clinical pathological features with a significant correlation between TP53 mutation and univariate analysis were

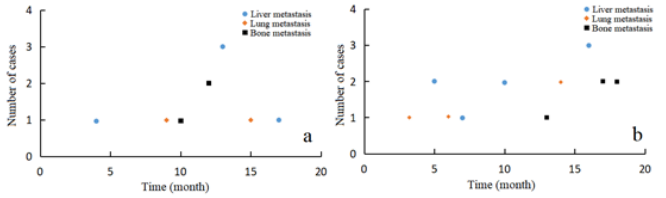

Figure 3. Comparison of transfer rate between patients with Mutant of TP53 gene and patients with wild-type (a.TP53 wild-type; b.TP53 Mutant).

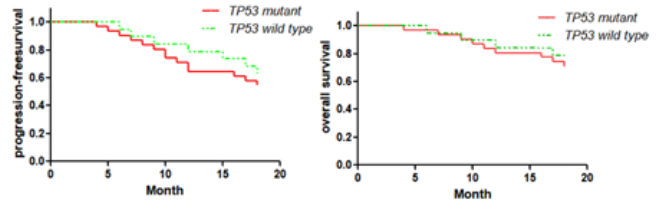

Figure 4. Comparison of survival between patients with TP53 gene Mutant and wild-type patients.

included in the multivariate analysis of survival prognosis. The results showed that there was no significant correlation between TP53 mutation and PFS (HR=0.67, 95% CI: 0.41-1.08, P=0.102), while TP53 mutation was a protective factor for OS (HR=0.49,95% CI: 0.27-0.90, P=0.022).

Discussion

Breast cancer is one of the most common gynecological malignancies in China, with a very high incidence rate. although the diagnosis and treatment methods are constantly updated, breast cancer is still one of the important causes of death. The occurrence and development mechanism of breast cancer is complex, and its process involves multiple genes, multiple steps, and multiple internal and external factors such as immunity, environment and genetics (12). Research shows that the disordered cell signal transduction pathway plays a very important role in the occurrence and development of breast cancer, and the mutation of related genes plays an important role in the progression, pathological classification and prognosis of breast cancer (13).

At present, the clinical use of gene mutation detection for breast cancer patients is mainly to obtain tDNA through tissue examination. However, when breast cancer is diagnosed as stage IIIB and IV, most of them cannot be operated on, and it is difficult to obtain tumor tissue. Moreover, the biological characteristics of the tumor may have changed after a series of treatments. Therefore, the real-time acquisition of tumor information before each treatment can more accurately reflect the characteristics of tumor cells, obtaining tissue samples again after the disease progresses can be even more difficult. Therefore, TP53 Genetic testing only through tDNA brings a lot of inconvenience to clinical treatment and research. People hope to obtain the patient's gene information through some non-invasive means.

The release of ctDNA in blood is believed to be related to the self-secretion, apoptosis, and necrosis of tumor cells. Meanwhile, due to the non-invasive nature of the detection, it can serve as a "liquid biopsy" and become a good prognostic and predictive molecular indicator. As early as 1948, French scholars Mandel et al. discovered

Table 3. Univariate and multifactorial COX survival analysis of PFS in the total population.

Item	Univariate analysis			Multivariate analysis		
	HR	95%CI	*P*	HR	95%CI	*P*
TP53 mutation	0.78	0.51~1.33	0.445	0.67	0.41~1.08	0.102
Age	1.64	1.01~2.79	0.032	2.06	1.25~3.39	0.005
Menstruation	1.39	0.91~2.38	0.110	NA	NA	NA
Tumour size	2.47	1.46~4.19	<0.001	2.11	1.23~3.61	0.007
Lymph node status	3.12	1.90~4.95	<0.001	2.48	1.49~4.13	<0.001
Pathology	1.42	0.62~2.93	0.443	NA	NA	NA
Histological grading	1.01	0.62~1.67	0.879	NA	NA	NA
Ki-67	0.79	0.47~1.31	0.294	NA	NA	NA
Vascular carcinoma thrombosis	3.43	1.89~5.61	<0.001	2.42	1.34~4.38	0.003
HRR mutation	0.71	0.39~1.34	0.227	NA	NA	NA

Table 4. Univariate and multivariate Cox survival analysis of OS in the general population.

Item	Univariate analysis			Multivariate analysis		
	HR	95%CI	*P*	HR	95%CI	*P*
TP53 mutation	0.58	0.33~1.08	0.087	0.49	0.27~0.90	0.022
Age	1.37	0.71~2.47	0.370	NA	NA	NA
Menstruation	1.81	0.95~3.41	0.072	NA	NA	NA
Tumour size	2.75	1.36~5.61	0.005	2.12	1.02~4.41	0.044
Lymph node status	6.10	2.92~12.71	<0.001	5.43	2.54~11.60	<0.001
Pathology	1.31	0.47~3.71	0.592	NA	NA	NA
Histological grading	1.21	0.52~2.00	0.946	NA	NA	NA
Ki-67	0.67	0.42~1.51	0.477	NA	NA	NA
Vascular carcinoma thrombosis	2.34	1.16~4.80	0.018	1.21	0.57~2.56	0.616
HRR mutation	0.82	0.40~1.75	0.635	NA	NA	NA

the presence of circulating DNA in human blood. It was not until 30 years later that Leon et al. reported for the first time that ctDNA levels in tumor patients were higher than those in normal individuals, and were related to the prognosis and efficacy of patients. Subsequently, it was found that elevated serum ctDNA levels were associated with tumor metastasis, clinical staging, and patient survival. The prognosis of patients can be determined based on serum ctDNA levels. High throughput sequencing technology can sequence hundreds to millions of DNA molecules at once and can detect gene mutations below 0.5%. It is a revolutionary change to traditional sequencing and requires a small sample size of DNA, making it suitable for the detection of trace amounts of DNA such as ctDNA.

This study used high-throughput sequencing technology to isolate peripheral blood ctDNA and perform multigene joint detection. The results showed that using tDNA detected by tissue paraffin sections as the gold standard, the sensitivity of plasma ctDNA was 75% to 100%, the specificity was 95.83% to 100%, and the overall coincidence rate with tDNA results was 90.32% to 100%. Among them, two patients did not detect mutations in their tissue sections, but TP53 exon5 deletion and TP53 exon8 mutation were detected in their respective blood samples, indicating that the gene mutations of the tumor may have changed during treatment, and the tissue sections cannot reflect the actual gene mutation situation before treatment. Alternatively, due to the heterogeneity of tumors, tissue samples cannot reflect the true gene expression due to limited sampling.

This study used high-throughput sequencing technology to isolate peripheral blood ctDNA and perform multigene joint detection. The results showed that using tDNA detected by tissue paraffin sections as the gold standard, the sensitivity of TP53 gene mutations in plasma ctDNA was 75% to 100%, the specificity was 92.31% to 100%, and the overall coincidence rate with tDNA results was 83.33% to 100%. Among them, two patients did not detect mutations in their tissue sections, but TP53 exon5 deletion and TP53 exon8 mutation were detected in their respective blood samples, indicating that the gene mutations of the tumor may have changed during treatment, and the tissue sections cannot reflect the actual gene mutation situation before treatment. Alternatively, due to the heterogeneity of tumors, tissue samples cannot reflect the true gene expression due to limited sampling. At the same time, the TP53 mutation status, clinicopathological characteristics and prognosis of 50 patients with breast cancer were analyzed retrospectively. The results showed that the overall mutation rate of TP53 was 51.67%, the most susceptible Exon to mutation was exon 7 Exon (23.40%), and the most common mutation type was Missense mutation (44.68%). Compared with TP53 Mutant patients, TP53 wild-type patients were younger at first diagnosis and had higher ER and PR positive rates. Bakhuizen et al. (14) conducted high-throughput sequencing on 370 breast cancer patients. TP53 was the gene with the highest mutation frequency, and the most common mutation region was located in the DNA binding domain; R175H, R273C/H, and R337L are the most common mutant subtypes. Yi et al. (15) conducted Genetic testing on circulating tumor DNA (ctDNA) in the plasma of 804 Chinese patients with breast cancer, and the results showed that the mutation frequency of TP53 was 53.6%. Riva et al. (16) conducted Exon sequencing on 46 triple-negative breast cancer patients and found that the mutation rate of TP53 was 86.96%. In this study, the mutation rate of this gene was 62.0%, similar to previous research reports (16). However, the mutation rate of genes is closely related to the state of the disease, the type of test specimen, and the treatment plan.

Survival analysis showed that there was no significant difference in the overall survival rate and progression-free survival rate of patients with TP53 Mutant ($P>0.05$). TP53 mutation is a protective factor that affects the overall survival rate, and TP53 is the most frequently mutated gene in cancer. Previous studies have reached different conclusions on the relationship between TP53 and prognosis in breast cancer patients. Basho et al. (17) found that TP53 mutation was significantly associated with shorter PFS and OS in patients with metastatic breast cancer ($P<0.001$). However, the study included patients with advanced breast cancer, suggesting that TP53 mutation may play different roles in the biological behavior of tumors at different stages. In addition, some studies have shown that TP53 mutation is a risk factor for poor prognosis in breast cancer patients, but whether TP53 mutation has a significant impact on survival in breast cancer patients receiving chemotherapy (15). In this study, 43 patients (96.58%) with breast cancer received neoadjuvant chemotherapy, which may partly explain that there is no statistically significant difference in PFS and OS between the two groups. In addition, the prognostic role of different mutation types of TP53 is not yet unified. In this study, there was no significant difference in the prognosis of breast cancer patients with TP53 Missense mutation and non-Missense mutation. In 2006, a study showed that among breast cancer patients with DBD mutations, there was no statistical difference between Missense mutation and other mutation types in the impact of TP53 on prognosis (18). The results showed that TP53 mutation in Exon 5~8 was an independent risk factor for DFS ($P=0.009$), but its prognostic role in the breast cancer subgroup was not clear (15, 19-27). Therefore, the impact of TP53 mutation on the prognosis of breast cancer patients remains to be further explored. Besides TP53, there may be other more important gene mutation-driven events.

Through this study, we found that high-throughput sequencing technology can detect extremely small amounts of ctDNA gene mutations in peripheral blood, and has a high coincidence rate with tDNA. Therefore, it is feasible to replace tumor tissue with TP53 gene mutations detected in peripheral blood, and it is expected to replace tissue slices that are limited by specimen collection, continuous monitoring, and follow-up. At the same time, this study retrospectively analyzed the Genetic testing results of 50 patients with breast cancer, explored the mutation spectrum of the TP53 gene, and the relationship between the gene mutation and clinical characteristics and prognosis. However, this is a retrospective study from a single center, with unavoidable selective bias. In addition, future large-scale and prospective studies are needed to validate the results of this study.

Conflict of interests

The authors declared no conflict of interest.

References

1. Harbeck N, Gnant M. Breast cancer. Lancet 2017; 389(10074):

1134-1150.
2. He A, Zhou T. Clinicopathological Features and Survival for Low ER-positive Breast-cancer Patients. Altern Ther Health M 2022; 28(6): 36-41.
3. Bouchelaghem R, Mader S, Gaboury L, Messarah M, Boumendjel M, Boumendjel A. Estrogens desensitize MCF-7 breast cancer cells to apelin-induced autophagy and enhanced growth under estrogen starvation: a possible implication in endocrine resistance. Cell Mol Biol 2022; 68(9): 113-124.
4. Yang Y, Zhu X, Xie Y, Wang X, Xie B, Zhang J. Metastasis to the Pancreas From Ductal Carcinoma In Situ of Breast Cancer: A Case Report and Review of Literature. Altern Ther Health M 2022; 28(6): 150-155.
5. Sukumar J, Gast K, Quiroga D, Lustberg M, Williams N. Triple-negative breast cancer: promising prognostic biomarkers currently in development. Expert Rev Anticanc 2021; 21(2): 135-148.
6. Kaur RP, Vasudeva K, Kumar R, Munshi A. Role of p53 Gene in Breast Cancer: Focus on Mutation Spectrum and Therapeutic Strategies. Curr Pharm Design 2018; 24(30): 3566-3575.
7. Sheikh A, Hussain SA, Ghori Q, et al. The spectrum of genetic mutations in breast cancer. Asian Pac J Cancer Prev 2015; 16(6): 2177-2185.
8. Duffy MJ, Synnott NC, Crown J. Mutant p53 in breast cancer: potential as a therapeutic target and biomarker. Breast Cancer Res Tr 2018; 170(2): 213-219.
9. Han SA, Kim SW. BRCA and Breast Cancer-Related High-Penetrance Genes. Adv Exp Med Biol 2021; 1187: 473-490.
10. Nong J, Gong Y, Guan Y, et al. Circulating tumor DNA analysis depicts subclonal architecture and genomic evolution of small cell lung cancer. Nat Commun 2018; 9(1): 3114.
11. Yi Z, Ma F, Liu B, et al. Everolimus in hormone receptor-positive metastatic breast cancer: PIK3CA mutation H1047R was a potential efficacy biomarker in a retrospective study. Bmc Cancer 2019; 19(1): 442.
12. Morales-Borges RH, Gonzalez MJ, Duconge J, Minich DM. N-Acetyl Cysteine and Glutathione in Health and Cancer-Pharmacogenomics, Research, and Clinical Practice: Hypothesis and Review. Altern Ther Health M 2022; 28(7): 169-177.
13. Kanabe BO, Ozaslan M, Aziz SA, Al-Attar MS, Kilic IH, Khailany RA. Expression patterns of LncRNA-GAS5 and its target APOBEC3C gene through miR-103 in breast cancer patients. Cell Mol Biol 2021; 67(3): 5-10.
14. Bakhuizen JJ, Hogervorst FB, Velthuizen ME, et al. TP53 germline mutation testing in early-onset breast cancer: findings from a nationwide cohort. Fam Cancer 2019; 18(2): 273-280.
15. Yi Z, Ma F, Rong G, Guan Y, Li C, Xu B. Clinical spectrum and prognostic value of TP53 mutations in circulating tumor DNA from breast cancer patients in China. Cancer Commun 2020; 40(6): 260-269.
16. Riva F, Bidard FC, Houy A, et al. Patient-Specific Circulating Tumor DNA Detection during Neoadjuvant Chemotherapy in Triple-Negative Breast Cancer. Clin Chem 2017; 63(3): 691-699.
17. Basho RK, de Melo GD, Ueno NT, et al. Clinical outcomes based on multigene profiling in metastatic breast cancer patients. Oncotarget 2016; 7(47): 76362-76373.
18. Olivier M, Langerod A, Carrieri P, et al. The clinical value of somatic TP53 gene mutations in 1,794 patients with breast cancer. Clin Cancer Res 2006; 12(4): 1157-1167.
19. Yang Y, Jiao Y, Mohammadi MR. Post-translational modifications of proteins in tumor immunotherapy and their potential as immunotherapeutic targets. Cell Mol Biomed Rep 2023 Feb 25. doi: 10.55705/cmbr.2023.378480.1091.
20. Ali Salman R. Prevalence of women breast cancer. Cell Mol Biomed Rep 2023 Mar 20. doi: 10.55705/cmbr.2023.384467.1095.
21. Kanwal N, Al Samarrai OR, Al-Zaidi HM, Mirzaei AR, Heidari MJ. Comprehensive analysis of microRNA (miRNA) in cancer cells. Cell Mol Biomed Rep 2023 Jun 1;3(2):89-97. doi: 10.55705/cmbr.2022.364591.1070
22. Li X, Mohammadi MR. Combined Diagnostic Efficacy of Red Blood Cell Distribution Width (RDW), Prealbumin (PA), Platelet-to-Lymphocyte Ratio (PLR), and Carcinoembryonic Antigen (CEA) as Biomarkers in the Diagnosis of Colorectal Cancer. Cell Mol Biomed Rep 2023 Jun 1;3(2):98-106. doi: 10.55705/cmbr.2023.374804.1088.
23. Alsaedy HK, Mirzaei AR, Alhashimi RA. Investigating the structure and function of Long Non-Coding RNA (LncRNA) and its role in cancer. Cell Mol Biomed Rep 2022 Dec 1;2(4):245-53. doi: 10.55705/cmbr.2022.360799.1062.
24. Alavi M, Rai M, Martinez F, Kahrizi D, Khan H, Rose Alencar De Menezes I, Douglas Melo Coutinho H, Costa JG. The efficiency of metal, metal oxide, and metalloid nanoparticles against cancer cells and bacterial pathogens: different mechanisms of action. Cell Mol Biomed Rep 2022 Mar 1;2(1):10-21. doi: 10.55705/cmbr.2022.147090.1023.
25. Alhashimi RA, Mirzaei A, Alsaedy H. Molecular and clinical analysis of genes involved in gastric cancer. Cell Mol Biomed Rep. 2021;1(3):138-46. Alhashimi, R. A., Mirzaei, A., Alsaedy, H. Molecular and clinical analysis of genes involved in gastric cancer. Cell Mol Biomed Rep 2021; 1(3): 138-146. doi: 10.55705/cmbr.2021.355860.1056.
26. Tourang M, Fang L, Zhong Y, Suthar RC. Association between Human Endogenous Retrovirus K gene expression and breast cancer. Cellular, Molecular and Biomedical Reports. 2021 Apr 1;1(1):7-13. Tourang, M., Fang, L., Zhong, Y., Suthar, R. Association between Human Endogenous Retrovirus K gene expression and breast cancer. Cell Mol Biomed Rep 2021; 1(1): 7-13. doi: 10.55705/cmbr.2021.138810.1008.
27. Bilal I, Xie S, Elburki MS, Aziziaram Z, Ahmed SM, Jalal Balaky ST. Cytotoxic effect of diferuloylmethane, a derivative of turmeric on different human glioblastoma cell lines. Cell Mol Biomed Rep 2021 Apr 1;1(1):14-22. doi: 10.55705/cmbr.2021.138815.1004.