Original Article

Molecular profiles and circulating tumor-DNA detected in Chinese early stage breast cancer

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Background: With the development of gene-sequencing technology, genome biomarkers, including Erb-B2 receptor tyrosine kinase 2 (ERBB2), phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha (pIK3CA), BReast CAncer gene 1 (BRCA1), and BReast CAncer gene 2 (BRCA2), and immunomarkers, including the tumor mutational burden (TMB) and programmed death-ligand 1 (PD-L1), have become important in the selection of treatment.

Methods: Twenty patients with early stage breast cancer who underwent surgery were enrolled in this study. Tissue samples and paired postoperative peripheral blood samples were collected and subjected to the targeted-capture sequencing of 1,021 cancer-associated genes.

Results: The most frequently altered genes were tumor protein 53 (*TP53*; 70%), *PIK3CA* (40%), protooncogene *MYC* (35%), *ERBB2* (30%), and cyclin-dependent kinase 12 (*CDK12*; 20%). Six (30%) patients presented with *ERBB2* amplification of NGS and simultaneously were positive for human epidermal growth factor receptor 2 (*HER2*) of IHC. *ERBB2* amplification and being *HER2* positive were common in breast cancer patients without lymph node metastasis (5/6, 83.3%) and those in stages IA–IIA. Most of the somatic mutations clustered in the *TP53* pathway, followed by the *PI3K* pathway. The TMB was lower than metastatic breast cancer in our cohort, and ranged from 0 to 9.6 mut/Mb (median: 1.92 mut/Mb). Interestingly, more patients had the *ERBB2* mutation in the non-lymph node metastasis group than the lymph node metastasis group (55.6% *vs.* 9.1%; P=0.049). Similarly, more patients had the *CDK12* mutation in the non-lymph node metastasis group than the lymph node metastasis group (44.4% *vs.* 0%; P=0.026). Circulating tumor deoxyribonucleic acid (ctDNA) was detected in 7 of the 20 patients (35%). Of these patients, 71.4% (5/7) were in stage I/II. In addition, no correlation was found between ctDNA detection and clinicopathological features or the driver gene mutations (e.g., *PIK3CA* and *ERBB2*). However, patients positive for ctDNA had a higher TMB than those negative for ctDNA when grouped according to the median TMB (1.92 mut/Mb; 85.7% *vs.* 38.5%; P=0.043).

Conclusions: This study described that genomic characteristics of Chinese early stage breast cancer, and the results showed that TMB was related to the detection of ctDNA in postoperative blood.

Keywords: Circulating tumor DNA (ctDNA); tumor mutational burden (TMB); lymph node metastasis; early stage breast cancer

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Introduction

This article provides an overview of the incidence and mortality of cancer in the world and China in 2020 based on the most recent data compiled by the International Agency for Research on Cancer (IARC). The IARC reported that female breast cancer has now surpassed lung cancer as the most common cancer in the world and China (1,2). Thus, more attention needs to be paid to its occurrence and development.

Breast cancer is a highly heterogeneous disease with diverse tissue morphology and molecular subtypes, each of which has different molecular characteristics, prognoses, and responses to therapies (3,4). Based on the expression of Ki67, 5 molecular types of breast cancer have been identified, and more subtypes remain to be reported (5,6). According to immunohistochemical or fluorescence *in situ* hybridization markers, breast cancer consists of the following 3 major tumor subtypes: estrogen receptor (*ER*) or progesterone receptor (*PR*) expression and Erb-B2 receptor tyrosine kinase 2 [*ERBB2*; human epidermal growth factor receptor 2 (*HER2*)] gene expression or amplification. Many studies have shown the that 3 subtypes have different prognoses and treatment strategies (7,8). However, with the development of gene-sequencing technology, more and more genome biomarkers [e.g., BReast CAncer gene 1 (*BRCA1*), BReast CAncer gene 2 (*BRCA2*), phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha (*PIK3CA*), and cyclin-dependent kinase 4/6 (*CDK4/6*)], and immunomarkers [e.g., tumor-infiltrating lymphocytes, and programmed death-ligand 1 (*PD-L1*)] have been reported (8).

8 targeted therapy drugs for metastatic breast cancer have been approved, including trastuzumab for *HER2*+ patients, and Olaparib for germline *BRCA* mutation patients (7). However, the treatments of early stage and locally advanced breast cancer mainly include surgical resection, neoadjuvant chemotherapy or adjuvant radiotherapy or chemotherapy (9). Unlike advanced breast cancer, early stage breast cancer focuses more on the risk assessment of postoperative recurrence and metastasis. Exploring the key mutation events in the occurrence and development of early breast cancer may be conducive to the discovery of biomarkers, which are beneficial to the early screening and prevention of breast cancer.

In relation to the genomic mutations, tumor protein (*TP53*) and *PIK3CA* have been implicated in the development of breast cancer (10). Some studies have reported differences in the genomic mutation profiles of breast cancer between Chinese and Western patients. Young Chinese breast cancer patients (aged ≤35 years old) have higher mutation rates of *TP53* (51% *vs.* 30%) and *ERBB2* (34% *vs.* 24%) than young Western breast cancer patients; however, the *PIK3CA* (25% *vs.* 30%) and *GATA3* (15% *vs.* 24%) mutation frequency is significantly lower in Chinese breast cancer patients than Western breast cancer patients (11).

Circulating tumor DNAs (ctDNAs) are some free DNA fragments produced by tumor cells in the process of necrosis or apoptosis. CtDNA carries the complete information of gene mutations in tumors or metastatic lesions, and these mutations will undergo some changes during treatment. Therefore, dynamic monitoring of ctDNA mutations can indicate treatment response to a certain extent. Based on high-depth and high-coverage of targeted next-generation sequencing (NGS), it can detect and analyze low-frequency variants of ctDNA. Therefore, the application of ctDNA to early detection and diagnosis of cancer is of great value. In breast cancer, recent studies have shown that ctDNA plays an important role in identifying micrometastasis and predicting the risk of recurrence, evaluating resistance to treatment or revealing tumor heterogeneity (12,13).

To better understand the genomic differences in breast cancer between Chinese and Western patients, we retrospectively analyzed the genomic data of tumor tissues and blood samples of 20 Chinese early invasive ductal breast cancer patients. Combined with the patients' clinical information, a comprehensive analysis was carried out based on tissue mutation maps, tumor signaling pathways, the factors affecting lymph node metastasis, and the detection of circulating tumor deoxyribonucleic acid (ctDNA) in postoperative blood. The results of our analyses expected to provide insights into early stage breast cancer. We present the following article in accordance with the MDAR reporting checklist (available at https://gs.amegroups.com/article/view/10.21037/gs-21-691/rc).

Methods

Study design and patients

The data of 20 breast cancer patients who underwent next generation sequencing (NGS) at Geneplus-Beijing (Beijing, China) were analyzed. The study was approved by the Ethics Committee of The First Affiliated Hospital of Soochow University (No. 2022013) , and each patient provided their informed consent. All procedures were

Table 1 Patient and tumor characteristics by study cohort

Characteristics	N=20
Age, median [range] years	42 [33–79]
Subtype	
HR+/HER2–	10 (50%)
TNBC	4 (20%)
HER2+	6 (30%)
Tumor size, median [range], cm	2.25 [1.3–5]
Stage	
I	6 (30%)
II	8 (40%)
III	6 (30%)
N stage	
N0	9 (45%)
N1/N2	11 (55%)
Family history	3 (15%)
Histological type	
Invasive ductal carcinoma	20 (100%)

HR+, hormone receptor positive; HER2 (also known as ERBB2), human epidermal growth factor receptor 2; TNBC, triple negative breast cancer.

conducted in accordance with the Declaration of Helsinki (as revised in 2013).

DNA sequencing

Paired pretreatment tissue samples and plasma samples were used to identify mutations with a NGS panel containing 1,021 cancer-associated genes. As previously reported (14), we use TruSeq DNA kits to prepare genomic DNA (gDNA) sequencing libraries. Subsequently, DNA sequencing was performed on the CN 500 sequencing instrument (Illumina, San Diego, CA, USA). The reads were aligned to the human genome build Genome Reference Consortium (GRC) h37 using a Burrows-Wheeler aligner (15). MuTect2 (3.4-46-gbc02625) was used to identify single nucleotide variants (SNVs) (16), while Genome Analysis Toolkit (GATK) was employed to identify small insertions and deletions (indels) (17). All final candidate variants were verified using an integrative genomics viewer browser. Tumor mutational burden (TMB) was calculated as the number of somatic non-synonymous SNVs (indels per Mb in the coding region, with a variant allele fraction of ≥0.03).

Postoperative blood samples were collected 3–7 days after surgery. The blood samples were collected in ethylenediaminetetraacetic acid vacutainer tubes (BD Diagnostics, NJ, USA) and were subjected to the laboratory process within 3 h. DNeasy 19 and QIAamp (Qiagen) kits were used to extract gDNA from leukocytes and circulating free DNA (cfDNA) from plasma, and then KAPA DNA kits (Kapa Biosystems, MA, USA) were used to construct sequencing libraries. Next, DNA sequencing was performed as described above.

Statistical analyses

Differences in patients' demographics were evaluated using the Fisher *t*-test. The other statistical analyses were performed using GraphPad Prism 8.0.2 (GraphPad Software, Inc.), and differences were evaluated using a 2-tailed unpaired Mann-Whitney U test. P values <0.05 were considered statistically significant.

Results

Patient cohort

Twenty patients, who had been diagnosed with breast cancer, were enrolled in this study. The characteristics of the patients are summarized in *Table 1*. The age of the patients ranged from 33 to 79 years (median: 42 years). 50% of patients had the hormone receptor positive (HR+) subtype, 4 patients had the triple-negative subtype, and 6 patients had the *HER2* positive subtype. Tumor size ranged from 1.3 to 5 cm. Patients were distributed across stages I/II/III and most patients had lymph node metastasis. 3 patients had a family history of breast cancer, but none of the patients had a family history associated with hereditary breast cancer ovarian cancer syndrome. All lesions were invasive ductal carcinomas. None of the patients had received any treatment other than surgery.

Molecular characteristics of patients

Primary tumor samples from the 20 patients were sequenced to identify somatic mutations. Mutations were identified in all patients. Seventeen patients (85%) had multiple mutations, and only 3 patients (15%) had 1 mutation. The median allele frequency was 15.7%;

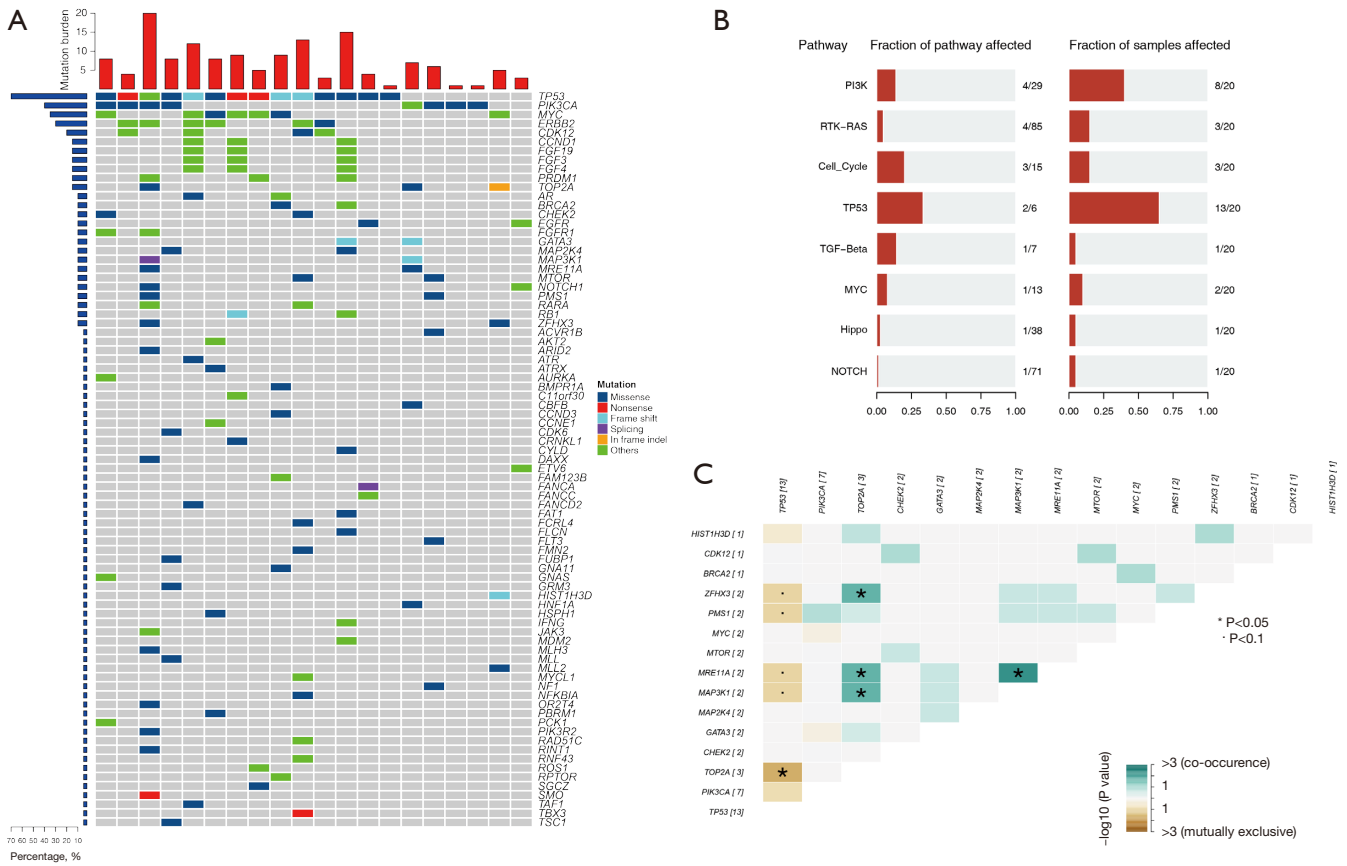

Figure 1 Genetic aberrations of breast cancers in a Chinese cohort. (A) Landscape of genetic alternations in 20 Chinese breast cancer patients. Top: the mutation numbers of each sample. Left: the mutation percentage of each gene in the total group. (B) Number of affected genes in difference pathways. (C) The co-occurrence of mutations in 20 patients.

142 somatic mutations were identified, including 77 somatic base substitutions, 12 small indels, 49 copy number variations and 4 rearrangements. As *Figure 1A* shows, the most frequently altered genes were *TP53* (70%), *PIK3CA* (40%), *MYC* (35%), *ERBB2* (30%), and *CDK12* (20%).

The consistency of *ERBB2* amplification with NGS with the results of clinical immunohistochemical detection of *HER2* was as high as 100%. Six (30%) patients were detected to have both *ERBB2* amplification and to be *HER2* positive, and these two markers were simultaneously detected were common in the breast cancer patients without lymph node metastasis (5/6, 83.3%) and in stages IA–IIA. In addition, *HER2*+ patients had a higher incidence of concomitant mutations than the HER2− patients, such as *MYC*, Cyclin D1 (*CCND1*), or fibroblast growth factor (*FGF*) family gene amplification, *PIK3CA* or *ERBB2* SNV. TMB-H was detected in 1 of the *HER2*+ patients, and another patient a novel gene rearrangement of *RARA-MYO15B*. Additionally, 1 patient with the triple-negative breast cancer (TNBC) subtype had 3 novel gene rearrangements, comprising *ETV6-PRB2* [intergenic, E26 transformation-specific variant 6 gene (ETV6), The retinoblastoma protein pRb and its relatives pRb2 (p130)], *NOTCH1-PMPCA* [intergenic, Notch is an ancient signaling pathway that includes Notch 1, mitochondrial processing peptidase-αprotein (PMPCA)], and *EGFR-LRP6*. Eight (40%) patients had the *PIK3CA* mutation (5 had the *HR*+, 2 had *HER2*+, and 1 had the TNBC subtype). Six patients had *PIK3CA* hotspot mutations in the helical domain (p.H1047R), and the other 2 mutations occurred in other domains (p.E418_L422delinsV and p.E545A).

A pathway analysis was conducted of all the mutant genes. Most of the mutated genes clustered in the *TP53* pathway, followed by the *PI3K* pathway. Among the genes involved in the *PI3K* pathway, the most commonly mutated

were *PIK3CA* (62%) and Mammalian target of rapamycin (*MTOR*) (15%). Among the genes involved in the *RTK/RAS* (Receptor Tyrosine Kinase/Rat sarcoma) pathway, the most commonly mutated were Mitogen-Activated Protein Kinase Kinase 4 (*MAP2K4*) (50%) and Mitogen-Activated Protein Kinase Kinase Kinase 1 (*MAP3K1*) (50%). Among the cell cycle pathway-related genes, the most commonly mutated were *MYC* (39%), *CDK12* (22%), and *CCND1* (17%; see *Figure 1B*). The co-existence of mutations detected in 20 patients was also analyzed. *TOP2A* was found to be mutually exclusive with *TP53* (P<0.05), and to co-occur with *MAP3K1*, Zinc Finger Homeobox 3 (*ZFHX3*), and MRE11 Homolog A (*MRE11A*) (P<0.05) (*Figure 1C*).

Next, the TMB was analyzed. A previous research study reported that the median TMB was 2.63 mut/Mb in primary or metastatic breast cancer (18). Another study reported that the mean TMB was 6.28 mut/Mb in advanced or metastatic breast cancer (19). However, in our cohort, the TMB was lower than metastatic breast cancer, and ranged from 0 to 9.6 mut/Mb (median: 1.92 mut/Mb). The TMB of the TNBC subtype was numerically, but not significantly, higher than the TMBs of the *HR*+ (4.8 *vs.* 0.96; P=0.315) and *HER2*+ (4.8 *vs.* 1.92; P=0.769) subtypes. Our results are consistent with those reported in another study (18).

Subsequently, the genomic characteristics were analyzed. Germline mutations were found in 3 (0.15%) patients, including 2 *BRCA2* mutations (1 pathogenic and 1 likely pathogenic) and 1 *RAD51D* pathogenic mutation. All 3 patients were young (aged 33, 45, and 47 years old) and 2 patients had TNBC subtypes. Additionally, 1 patient had a family history (that patient's father had a stomach or gastroesophageal junction tumor). Of these 3 patients, ctDNA was detected in 1 patient's postoperative peripheral blood sample.

Correlation between driver mutation and clinical characteristics

The therapeutic targeting of abnormal cancer driver mutations is garnering increased attention. Tumors with driver mutations were profiled in terms of their clinical and genomic characteristics. *PIK3CA* is a common driver mutation in breast cancer, especially in the *HR*+ subtype. As stated above, 8 of the 20 (40%) patients in this study presented with the *PIK3CA* mutation. The patients were divided into a *PIK3CA* mutation group and a wildtype group. The *PIK3CA* mutation group had a higher age (47 *vs.* 40; P=0.14) and a higher proportion of the patients in the *PIK3CA* mutation group were in stage I/II (85.7% *vs.* 69.2%; P=0.24) compared to the wildtype group; however, the differences were not significant. The *PIK3CA* wildtype group had a numerically higher proportion of patients with lymph node metastasis (61.5% *vs.* 42.9%; P=0.62) and a higher TMB (2.5 *vs.* 2; P=0.28) than the mutation group; however, the difference was not significant.

The relationship between the *ERBB2* mutation and clinical and genomic characteristics were also analyzed. The *ERBB2* mutation group had a numerically higher age (60 *vs.* 40; P=0.15), a higher TMB (2.4 *vs.* 1.4; P=0.51), and a higher proportion of patients in I/II stage (100% *vs.* 42.9%; P=0.26) compared to the wildtype group. Notably, the *ERBB2* wildtype group had a significantly higher proportion of patients with lymph node metastasis than the *ERBB2* mutation group (71.4% *vs.* 16.7%; P=0.049).

The essential role of *BRCA1* and *BRCA2* proteins in homologous recombination repair (*HRR*), which is a high-fidelity DNA double-strand break (DSB) repair mechanism, has been extensively documented. In this study, we also analyzed the *HRR* mutation. *HRR* mutations were found in 12 patients (5 had the *HR*+ subtype, 2 had the TNBC subtype, and 5 had the *HER2*+ subtype). No association was found between *HRR* mutations and clinical or genomic characteristics.

Genomic differences between patients with and without lymph node metastasis were then compared. *Figure 2* sets out the top 10 frequently altered genes in the 2 groups. The *TP53* and *PIK3CA* genes were highly altered in both groups. Further, both *ERBB2* mutations (55.6% *vs.* 9.1%; P=0.049) and high-frequency *CDK12* mutations (44.4% *vs.* 0%; P=0.026) were found in patients without lymph node metastasis, significantly difference to patients with lymph node metastasis.

Correlation between ctDNA and patient characteristics

Postoperative blood was obtained from all patients, and plasma DNA was extracted to analyze the presence of ctDNA. Buffy coat DNA was analyzed to control for the clonal hematopoiesis of indeterminate potential. ctDNA was detected in 7 of the 20 patients (35%), at a median allele frequency of 0.63%. Previous studies have shown that the detection of ctDNA in postoperative blood is associated with a higher risk of recurrence for early breast cancer (20,21). Thus, we investigated whether ctDNA detection was associated with any characteristics at diagnosis.

As *Table 2* shows, age and subtype were not associated

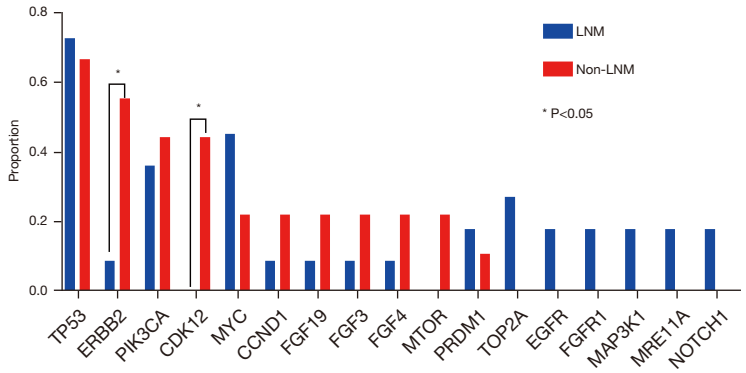

Figure 2 Correlation between lymph node metastasis and mutations. LNM, lymph node metastasis.

with ctDNA detection. Next, tumor size was compared in both the positive and negative groups. Interestingly, the majority of patients in whom ctDNA was detected were in stage I/II (71.4%). Similarly, driver mutations were not associated with the detection of ctDNA; 85.7% and 71.4% patients detected to have ctDNA had *PIK3CA* or *ERBB2* wildtype, respectively. *HRR* mutations may be associated with ctDNA detection, but the association was not significant (P=0.085). Subsequently, we observed that patients positive for ctDNA had a higher TMB when grouped by the median TMB (1.92 mut/Mb) than those negative for ctDNA. We also found that patients positive for ctDNA had a higher TMB than patients negative for ctDNA (85.7% *vs.* 38.5%; P=0.043). These results demonstrated that the detection of ctDNA in postoperative blood is not associated with clinical characteristics but is associated with the TMB of patients.

Discussion

Breast cancer is the most frequently diagnosed cancer worldwide (22). Approximately 95% of women presenting with early stage breast cancer do not have macroscopic metastatic disease (22). In the preceding decades, molecular characterization has revolutionized breast cancer research and therapeutic approaches. In this study we examined the genomic characteristic of Chinese early stage breast cancer Patients. Additionally, the relationship between ctDNA detection in postoperative blood and patients' characteristics was also described.

As Xiao *et al.* reported, *TP53* (47.0%), *PIK3CA* (45.0%), and *ERBB2* (30.0%) are the most frequently mutated genes, but other gene variations, such as *CDK12* (18.0%), also occur (11). In our study, the most frequently altered genes were *TP53* (70%), *PIK3CA* (40%), *MYC* (35%), *ERBB2* (30%), and *CDK12* (20%). Overall, the detection rate of high-frequency mutation genes in early breast cancer was relatively consistent. Xiao *et al.* also found that *ERBB2* aberrations were more likely to co-occur with *CDK12* [odds ratio (OR) =10]. Similarly, 25% (5/20) of patients were found have both the *ERBB2* and *CDK12* aberrations in our study.

Breast cancer is not a single entity; rather, it comprises multiple subtypes, each with its own set of genomic characteristics and signatures (23). The *PI3K* pathway is commonly altered in breast cancer and contributes to the development of breast cancer (24). In our cohort, 40% of patients had *PI3K* pathway mutations. Consistent with previous findings (22), *TP53* was the most common mutation in this study, followed by *PIK3CA*. The *PIK3CA* gene encodes for the α-isoform of the catalytic subunit (p110α) of class IA *PI3K* kinase. *PIK3CA* somatic mutations have been reported in around 20–40% of early breast cancer patients (25,26). In this study, 40% of the patients had *PIK3CA* mutations. Additionally, similar to previous studies (27), we found that the hotspot of *PIK3CA* was p.H1047R. As André *et al.* (28) reported, switching from *PIK3CA*-wildtype to *PIK3CA* mutations was very rare, which could explain the stable incidence between early and late stage breast cancer. Further, another study showed that *PIK3CA* mutations were associated with older age (29). However, while we found that patients *PIK3CA* mutations were somewhat older, they were not significantly older than the other patients.

Genomic features were compared between patients with and without lymph node metastasis. Interestingly, we found a higher proportion of *ERBB2* and *CDK12* mutations in patients without lymph node metastasis. However, a study

Table 2 Clinicopathologic and genomic factors associated with ctDNA detection

Characteristics	Positive (n=7)	Negative (n=13)	P value
Age, years			
Median	43	40	0.7123
Mean ± SD	47.5±4.83	46±3.71	
>42 year, n (%)	4 (57.1)	6 (46.2)	1
Subtypes, n (%)			0.376
Luminal A	0 (0)	3 (23.1)	
Luminal B	2 (28.6)	5 (38.4)	
HER2+	3 (42.8)	3 (23.1)	
TNBC	2 (28.6)	2 (15.4)	
Tumor size			
Median	2.3	2.2	0.876
Mean ± SD	2.54±0.46	2.72±0.32	
>2.25 cm, n (%)	4 (57.1)	6 (46.2)	1
Stage, n (%)			0.612
I/II	5 (71.4)	9 (69.2)	
III	2 (28.6)	4 (30.8)	
N-stage, n (%)			1
N0	3 (42.9)	6 (46.2)	
N1/N2	4 (57.1)	7 (53.8)	
PIK3CA, n (%)			0.328
mut	1 (14.3)	6 (46.2)	
wt	6 (85.7)	7 (53.8)	
ERBB2, n (%)			1
mut	2 (28.6)	3 (23.1)	
wt	5 (71.4)	10 (76.9)	
HRR, n (%)			0.085
mut	6 (85.7)	6 (46.2)	
wt	1 (14.3)	7 (53.8)	
TMB			
Median	2.88	0.97	0.17
Mean ± SD	3.84±1.19	2.58±0.81	
>1.92 mut/Mb, n (%)	6 (85.7)	5 (38.5)	0.0427

HER2 (also known as ERBB2), human epidermal growth factor receptor 2; TNBC, triple negative breast cancer; mut, mutation; wt, wild type; HRR, homologous recombination repair; TMB, tumor mutational burden.

of 589 Chinese patients with early stage breast cancer showed *ERBB2* and *CDK12* gene amplification were more enrichment in the lymph node metastasis group (11). Our study has some limitations. The sample size limited our analysis. Further, this single-center study, which was conducted in the Suzhou province, only included patients from southern China, which may have resulted in patient selection bias. Further research needs to be conducted to verify our findings. Specifically, a multi-center study should be conducted throughout China with a larger cohort.

In addition to TNBC, *HR*+ and *HER2*+ breast cancers are considered immunologically "cold" due to their low tumor-infiltrating lymphocyte infiltration (30). Additionally, the efficacy of single-agent therapy with pembrolizumab was evaluated in *ER*+/*HER2*– advanced breast cancer. Only 20% of *ER*+ breast cancer cells express the *PD-L1* immune-checkpoint protein, and single-agent immune-checkpoint inhibitors (ICIs) have shown limited efficacy (objective response rate: 12%, 3/25) in treating *ER*+ tumors (31). Previous studies have shown that TNBC has a higher *PD-L1* expression; however, only about 10% of breast cancers show *PD-L1* expression. Compared to other tumor types, *PD-L1* expression is not a valid predictive biomarker of ICI efficacy in breast cancer.

In recent years, the TMB has become a prominent independent efficacy prediction biomarker of ICIs in several cancer types, including lung cancer, melanoma, and endometrial cancer; however, the significance of the TMB in breast cancer remains unclear (32). Barroso-Sousa *et al.* reported that high TMB (18%) was associated with significantly longer PFS (12.5 *vs.* 3.7 months; P=0.04) in patients with metastatic TNBC (mTNBC) which received anti-PD-1/L1 inhibitors alone or combined with targeted therapy or chemotherapy (33). In another study, an immune-related prognostic score was established in 22 breast cancer cohorts with a total of 6,415 samples and developed a prognostic scoring system indicative of immune infiltration. Fifty-four immune cell types with the P<0.05 were identified as prognostic factors. Of these, 51 immune cell types with the HR <1 indicated that the higher levels of normalized enrichment score (NES) values were associated with longer survival time (34). One study enrolled 5,112 patients for RNA analysis to quantify 22 tumor-infiltrating immune cells (TIICs) in primary BC. The immune cell infiltration-based immune score model can be effectively and efficiently used to predict the prognosis of BC patients as well as the effect of chemotherapy (35). A study demonstrated that the median TMB was 2.63 mut/

Mb; however, the TMB varied significantly according to the tumor subtype (*HR–/HER2–* > *HER2+* >*HR+/HER2–*; P<0.05) and sample type (metastatic > primary; $P=2.2\times10^{-16}$) in primary (2,455/3,969, 61.9%) or metastatic (1,496/3,969, 37.7%) breast cancer (18). In advanced or metastatic breast cancer, the mean TMB was 6.28 mut/Mb (19). However, for early stage breast cancer, a study of Chinese breast cancer patients revealed that the median value of the TMB level was 4 mutations/Mb, and only 5.7% of patients had a TMB ≥10 mutations/Mb (11). In our study, a lower TMB level was observed; the median TMB was only 1.92 mut/Mb. However, the number samples in our study was small. Additionally, differences in the targeted-sequencing panel and the calculation method used to determine the TMB may have led to biases and inconsistencies in the data results. In all subtypes, the TNBC subtype had a numerically higher TMB than other subtypes; however, the differences were not significant. The correlation between driver mutations and the TMB was also analyzed. However, as previously reported (36), no associations were found between the TMB and *PIK3CA*, *ERBB2*, and *HRR* mutations.

Recently, ctDNA has been suggested to be a tumor biomarker because of its sensitivity, stability and specificity. However, the best method to measure ctDNA is uncertain. Digital PCR (dPCR) can only measure one or few known mutations. But NGS can measure many mutations simultaneously and can identify resistant clones newly emerged during treatment (13). An early lung cancer ctDNA detection study showed that, excluding technical limitations, there are mainly two factors that affect the consistency of ctDNA and tumor tissue detection. On the one hand, biological factors, such as the blood state of ctDNA before surgery, are easier to detect for Shedder. The pathological type such as lung squamous cell carcinoma is easier than lung adenocarcinoma. The higher Ki67, the easier to release DNA. Patients with lymphatic vascular invasion of the tumor are more likely to detect ctDNA. On the other hand, it is related to clinical treatment, such as detection time after surgery and after radiotherapy or chemotherapy. DYNAMIC studies have shown that the detection of ctDNA at 3 days after surgery is more likely to indicate recurrence than at 1 day after surgery (37). One of our study used the 1,021 panel have confirmed that blood ctDNA is highly consistent with somatic mutations in tumor tissues in patients with early breast cancer, and demonstrated that ctDNA can be used to predict tumor response to neoadjuvant chemotherapy (NAC) and prognosis (13). In addition, ctDNA can be used to predict and monitor therapeutic response in advanced breast cancer. Reported studies have confirmed that molecular tumor burden index (mTBI) indicators based on ctDNA detection can be used as predictors of disease progression. The results show that mTBI can evaluate the therapeutic efficacy and clinical imaging of 11 patients with metastatic breast cancer, and the mTBI of 6 patients was 8–16 weeks earlier than imaging, suggesting clinical disease progression (38). Another study has demonstrated that plasma PIK3CA ctDNA mutation is associated with clinical outcome in advanced breast cancer. The ctDNA analysis found *PIK3CA* p.H1047R mutation was more frequent in *HER2+* disease and associated with worse OS. It was also the only mutation associated with shorter PFS through a multivariate analysis of HER2+ patients who were treated with trastuzumab (39). In the ctDNA study for breast cancer, researchers evaluated the possibility of using plasma ctDNA to monitor minimal residual disease (MRD) in breast cancer. The studies have found that continuous monitoring of gene mutations in plasma improves the sensitivity of predicting recurrence, and can predict recurrence 7.9 months earlier than clinical testing. Compared with primary tumor tissue sequencing, deep sequencing of plasma ctDNA can more accurately predict metastasis and recurrence (40). Takeshita *et al.* analyzed 253 plasma samples from 119 HR-positive breast cancer patients, and detected cfDNA before, after, and during follow-up. It was found that patients with an increased frequency of *ESR1* gene mutations in cfDNA before and after treatment progressed in a shorter treatment time. ESR1 gene mutation is an effective predictor of treatment effect for hormone-positive metastatic breast cancer patients (41). As a biomarker, we found an association between the detection of ctDNA and a higher risk of recurrence for early breast cancer patients (20,21). We also found that the presence of ctDNA was not associated with the characteristics of patients. In particular, ctDNA was not more likely to be detected in patients with stage III than patients with I/II (10% *vs.* 35%). As previously reported (42), pre-operative ctDNA positivity of gastric cancer was associated with disease stage, and the number of patients that were ctDNA positive in stage III was greater than that in stages I and II (68% *vs.* 21%; P=0.0044). Patients with a higher tumor stage or lymph node involvement were more likely to have detectable ctDNA (P=0.005 and P=0.029, respectively). In addition, tumor volume was associated with ctDNA positivity (median 9 cm^3 in the ctDNA positive group *vs.* 4.5 cm^3 in the

ctDNA negative group; P=0.0582). In our study, the tumor volume of 1 stage IIA patient, whose postoperative ctDNA was positive, was 12.5 cm^3. Few studies have examined the relationship between postoperative ctDNA and early stage breast cancer. However, the TMB was found to be related to ctDNA detection. This study had a few limitations. The association between survival and ctDNA needs to be further analyzed. We plan to reanalyze our data when the postoperative follow-up data are more mature. Further research needs to be conducted to verify our findings with a larger cohort.

In conclusion, our study described that genomic characteristics of early stage breast cancer in Chinese patients. We found that the TMB was related to the detection of ctDNA in postoperative blood. Our findings provide novel insights into the characteristics related to genetic mutations and postoperative ctDNA.

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Footnote

Reporting Checklist: The authors have completed the MDAR reporting checklist. Available at https://gs.amegroups.com/article/view/10.21037/gs-21-691/rc

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Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at https://gs.amegroups.com/article/view/10.21037/gs-21-691/coif). MXZ is an employee of Geneplus-Beijing. The other authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. All procedures were conducted in accordance with the Declaration of Helsinki (as revised in 2013). The study was approved by the Ethics Committee of The First Affiliated Hospital of Soochow University (No. 2022013) . Written informed consent was provided by all the participants in the study.

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