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ORIGINAL RESEARCH

The distinct clinicopathological and prognostic implications of *PIK3CA* mutations in breast cancer patients from Central China

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Purpose: The mutation status and prognostic value of *PIK3CA* in breast cancer were widely investigated, which showed significant difference among the patients from vast areas around the world. In this study, the frequency, distribution, bias, and burden of *PIK3CA* mutations and their relationships with clinicopathologic variables and prognostic significances were investigated in the breast cancer patients from Central China.

Materials and methods: Somatic mutations in exon 9 and exon 20 of *PIK3CA* gene were analyzed using Sanger sequencing combining with targeted next generation sequencing in 494 breast cancer patients from Central China. The correlations between *PIK3CA* mutations and clinicopathological characteristics and the prognostic values of multiple *PIK3CA* mutation statuses were evaluated.

Results: *PIK3CA* mutations were found in 38% of the patients and associated with estrogen receptor-positive, progesterone receptor-positive, low Ki67 labeling index, and luminal/human epidermal growth factor receptor 2-enriched subtypes. Meanwhile, the prognosis of the total patients and the patients in old diagnostic age, progesterone receptor-negative, low Ki67 labeling index, and luminal/human epidermal growth factor receptor 2-enriched subgroups was significantly related to *PIK3CA* mutations. Most interestingly, the distribution, bias, and burden of *PIK3CA* mutations were correlated with different clinical, pathological, and molecular features as well as distinct prognostic implications in multiple breast cancer subgroups.

Conclusion: The frequency, distribution, bias, and burden of *PIK3CA* mutations were associated with various clinical, pathological, and molecular characteristics in the breast cancer patients from Central China. These different mutation statuses can be used as potential indicators of prognosis in multiple breast cancer subgroups.

Keywords: breast cancer, *PIK3CA*, clinicopathological characteristics, prognosis

Introduction

Breast cancer is the most diagnosed female cancer and the fifth leading cause of cancer death, and the mortality rate is 70,700 patients every year in China.[1] The incidence and mortality of this cancer in women is increasing in recent years.[1] Classical therapeutic strategy for this disease in clinic is combining the loco-regional therapy (surgery and radiation) with subsequent adjuvant systemic therapy.[2–4] However, as a highly heterogeneous disease, the therapeutic effect of breast cancer is determined by various classical clinical phenotypes (age at diagnosis, tumor size, stage of tumor, lymph node invasiveness, etc.) as well as intrinsic molecular subtypes (triple negative, human epidermal growth factor receptor 2 [HER2]-enriched, luminal A and luminal

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B subtypes).[2,5] Besides, in the era of personalized medicine, genetic factors get increasing attention, which are considered as the most important aspects in tumor initiation, progression, prognosis, and drug resistance.[4,6] Therefore, accurate molecular diagnosis of specific biomarkers that can respond to and monitor the therapeutic effect of breast cancer is essential.

As a major component of PI3K/AKT/mTOR signaling pathway, the activation of phosphatidylinositol-3 kinases (PI3K) which interacts with transmembrane tyrosine-kinase growth factor receptors subsequently activates AKT, mTOR, MAPK signaling pathways, and plays essential roles in multiple cellular processes including translation regulation, protein synthesis, cell metabolism, autophagy, cell adhesion, and apoptosis.[7,8] Numerous studies illustrated that hyperactivation of PI3K signaling intimately was associated with pathogenesis and progression of various types of cancers.[9–13] There are two most important events that could constitutively activate the PI3K signaling pathway – loss of the function of tumor suppressor phosphate and tension homology deleted on chromosome ten (PTEN) and activation mutations in p110α catalytic subunit which encoded by the *PIK3CA* gene.[8,14] Between them, *PIK3CA* is one of the most prevalent mutated genes in breast cancer even though its mutation frequency varies among different investigations.[15–19] According to the Catalogue of Somatic Mutations in Cancer Database (COSMIC, https://www.sanger.ac.uk), >90% of the *PIK3CA* mutations are located in the helical (exon 9) or kinase (exon 20) domains, including the hotspot mutations E542K, E545K in exon 9 and H1047R, H1047L in exon 20.

Therefore, multiple studies have been performed to investigate the relationship between *PIK3CA* mutations and clinicopathological features, prognostic value, or therapeutic relevance of breast cancer in different countries and races.[15,20–22] However, the results are controversial among these different studies even those from the same country. For example, in two investigations in the patients from Italy, Buttitta et al[23] showed that *PIK3CA* mutations were associated with HER2-negative clinicopathological subtype, and in contrast, no association was determined by Barbareschi et al.[24] Harlé et al[25] attempted to correlate the *PIK3CA* mutations with low-grade tumors in breast cancer patients from Nancy, France, while by investigating the patients from Saint-Cloud, France, Cizkova et al[26] believed that there were correlations between *PIK3CA* mutations and estrogen receptor (ER)-positive, progesterone receptor (PR)-positive, HER2 negative, low tumor grade or small tumor size. Considering the small sample sizes, population-related peculiarities of patients, and different methods used for detection of the mutations, these phenomena might demonstrate that it is crucially important to analyze carefully these factors in more areas including a larger population with a unique method. The outcomes would help us to deeply understand breast cancer and offer us specific and predictive biomarkers to be used for breast cancer diagnosis and treatment.

In this study, we investigated the frequency, distribution, bias, and burden of *PIK3CA* mutations in 494 breast cancer patients from a single center located in Central China and explored their associations with clinicopathological features and disease prognosis. These data will expand the knowledge of *PIK3CA* mutations related to breast cancer, which can be further used to provide precision medicine strategies to the breast cancer patients in Central China.

Materials and methods

Patients

The study was conducted in accordance with the Declaration of Helsinki. After obtaining written informed consent from all the patients and approval of the Ethics Committee of the First Affiliated Hospital of USTC, 537 formalin-fixed, paraffin-embedded (FFPE) primary breast tumor tissue samples were collected at the Department of Pathology, the First Affiliated Hospital of USTC, Division of Life Sciences and Medicine, University of Science and Technology of China from 2010 to 2017. Ultimately, 494 samples were eligible for our analysis and the clinical characteristics of these patients are summarized in Table S1. All the samples were obtained from female patients who did not receive preoperative treatment. Based on the American Joint Committee on Cancer TNM system (2010),[27] the pathological diagnosis of each sample was made by at least three pathologists. The Nottingham Prognostic Index (NPI)[28] was calculated to determine the prognosis of the patients after surgery using the following formula: NPI =0.2×tumor size (cm)+ grade (1–3)+ lymph node status (1–3). Progression-free survival (PFS) was defined as the time span between surgery date and the first relapse time of tumor, second primary tumor, death, or last follow-up.[29] Among 494 patients, 303 PFS and overall survival (OS) data were collected in which 28 of them had relapsed tumor or second primary tumor and 46 patients died. The follow-up period was from 5 to 97 months with a median time of 35 months.

Molecular subtypes of breast cancer

ER, PR, and HER2 statuses were determined by immunohistochemical (IHC) staining. ER or PR was regarded as positive when >1% of tumor cells were stained based on the St. Gallen International Expert Consensus.[30] HER2 was

considered positive when complete or intense membrane staining was determined in >30% of tumor cells. The subtypes of samples were classified using anatomopathological classification according to St. Gallen International Expert Consensus.[30] The expression of Ki67 – a cellular marker for proliferation – was also examined by IHC in all patients.

DNA preparation and targeted next generation sequencing

Tumor content of >50% was qualified through H&E staining and selected for DNA extraction. Genomic DNA was extracted from one 10 μm section using the GeneRead DNA FFPE Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. The quantity and quality of extracted DNA were determined using Nanodrop spectrophotometer 2000 (Thermo Fisher Scientific, MA, USA) and Qubit 3.0 (Life Technologies, Carlsbad, CA, USA).

Genetic variants of 24 samples were screened with TruSeq Amplicon Cancer Panel (Illumina, San Diego, CA, USA) using the NextSeq 500 sequencing system (Illumina). Mutations in 212 amplicons from 48 genes were examined in these samples, including *BRAF*, *KRAS*, *EGFR*, *TP53*, *NRAS*, *ALK*, *IDH1*, *FGFR*, *PTEN*, *RB*, *ATM*, *PIK3CA*, and other important cancer-related genes. Among them, the total 20 exons of *PIK3CA* were targeted.

After sequencing, mapping, and alignment, single nucleotide variants (SNVs) and indels were called and annotated based on the method described by The Cancer Genome Altas.[31] Variants with insufficient coverage (minimum depth of coverage <8) and low variant allele frequency (<0.03) were filtered out.

PCR amplification of the *PIK3CA* exon 9 and exon 20 fragments

The exon 9 and exon 20 of *PIK3CA* gene were amplified using the following primers: exon 9 forward 5′-TGTAAAACGACGGCCAGTCAGAGTAACAGACTAGCTAGAGACAATG-3′, exon 9 reverse 5′-CAGGAAACAGCTATGACCAATCTCCATTTTAGCACTTACCTGTGAC-3′, and exon 20 forward 5′-TGAGCAAGAGGCTTTGGAGTAT-3′, exon 20 reverse 5′-CCTATGCAATCGGTCTTTGC-3′. The PCR was performed in a 20 μL reaction system using FastStart Essential DNA Green Master Kit (Roche, Mannheim, Germany) with 1 μL gDNA, 1 μL forward primer (10 nmol/mL), 1 μL reverse primer (10 nmol/mL), and 10 μL FastStart Essential DNA Green Master. The PCR was carried out on a LightCycler 96 Real-Time PCR System (Roche) under the following conditions: initial denaturation at 95°C for 5 minutes, then 45 cycles of 95°C for 20 seconds, 60°C for 30 seconds, and 72°C for 30seconds.

Detection of *PIK3CA* mutations by Sanger sequencing

For sequence analysis, PCR products were purified by PCR Product Purification Kit (Generalbiol, Anhui, China) and subjected to bidirectional dye-terminator sequencing using M13 forward primer 5′-TGTAAAACGACGGCCAGT-3′ for exon 9 amplicon and exon 20 reverse primer 5′-CCTATGCAATCGGTCTTTGC-3′ for exon 20 amplicon. Sequencing fragments were detected by capillary electrophoresis using the ABI 3730xl DNA analyzer (Applied Biosystems, Carlsbad, CA, USA) and then analyzed by SnapGene Viewer 4.2.6. The mutations were identified by manual review.

Statistical analysis

The relations between *PIK3CA* mutation statuses and clinicopathological characteristics were assessed by the Pearson chi-squared tests using SPSS software v19.0.0 (IBM, NY, USA). The HR of *PIK3CA* mutation as well as clinicopathological variables was calculated by the Cox proportional hazards regression model in univariate analysis. Based on the PFS in different mutation conditions, the Kaplan–Meier survival curves were drawn using GraphPad Prism software v5.01 (GraphPad, San Diego, CA, USA) and the significant differences were displayed by the log-rank test (SPSS v 19.0.0, IBM). Statistical significance was considered as $P<0.05$.

Results

Somatic mutations in breast cancer patients from Central China

To investigate the somatic mutations in breast cancer, 24 primary tumor samples were analyzed using targeted next generation sequencing (NGS). A mean coverage depth of 8,525× was achieved. 94.4% of amplicons were covered at >500× depth. After SNV and indel calling, a total of 93 mutations were detected, including 27 synonymous SNVs, 48 nonsynonymous SNVs, 3 stopgains, 3 splicings, 10 frameshift deletions, 1 nonframeshift deletion, and 1 frameshift insertion. The overall mutation frequency was 2.75 nonsilent mutations (range of 0–12 mutations) per sample. As shown in Figure 1, the most frequently mutated gene was *TP53* (41.7%, 10 of the 24 patients) with various mutation types (missense mutation, nonsense mutation, frameshift insertion, and splicing). *PIK3CA* mutation was found in 33% of the samples, which ranked as the second highest mutated gene. All of the *PIK3CA* mutations were

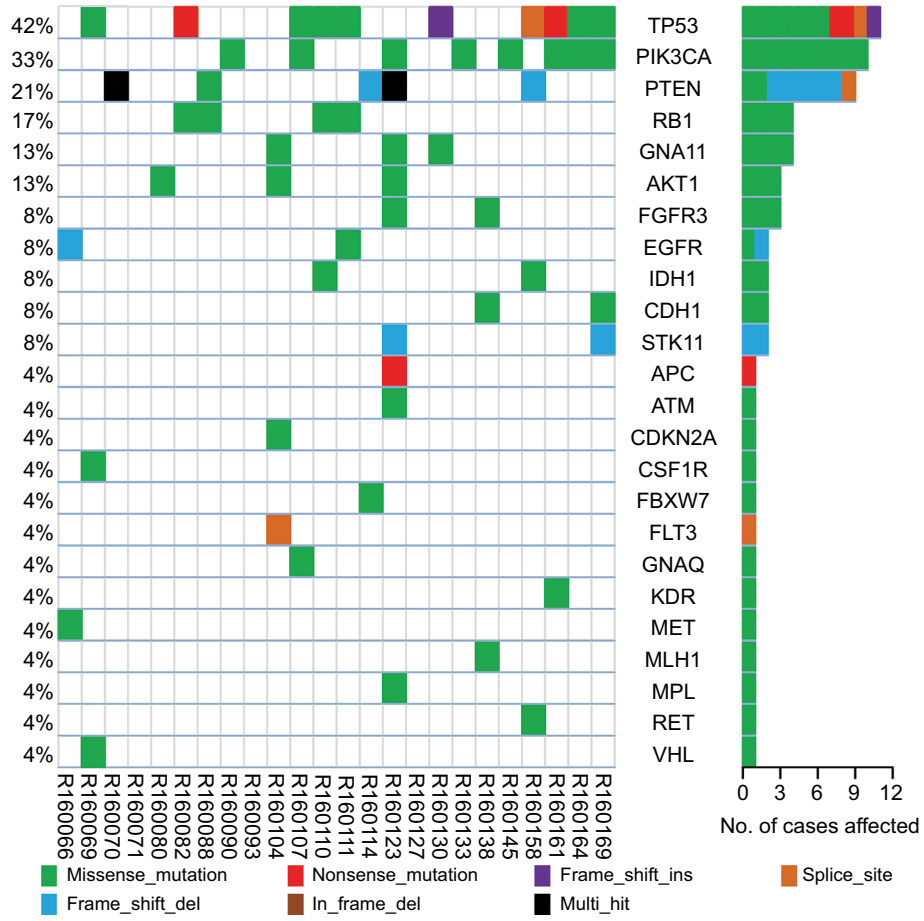

Figure 1 Somatic mutations of 24 significant mutated genes in 24 breast cancer patients.
Notes: The figure shows an overview of genomic alterations (right legend) in particular genes (rows) affecting individual samples (columns). The missense mutation, nonsense mutation, frame shift insertion, frame shift deletion, in frame deletion, splice site mutation, and multiple mutation are shown as green, red, purple, blue, brown, yellow, and black, respectively. Left legend denotes the percentage of mutations in 24 breast cancer samples and right plot represents the total number of mutations for each gene.

hotspot mutations including three E542K, three E545K, and two H1047R (Table S2). This much higher mutation rate in *PIK3CA* exon 9 (75%) in breast cancer was quite different from previous reports[19,32–34] which attracted our attention. Besides, we examined the clinicopathological variables of these patients and found that the samples harbored exon 9 mutations were HER2-negative tumors and all belonged to luminal B molecular subtype (Table S2). Furthermore, the only two tumor-relapsed cases were also from the group with exon 9 mutations, which demonstrated that exon 9 mutations might be related to poor prognosis (Table S2). Therefore, we hypothesized that the proportion of *PIK3CA* exon 9 mutations was much higher in the female breast cancer patients from our area (Central China) than the other regions, and this preference was correlated with special clinicopathological characteristics and tumor prognosis.

PIK3CA mutations detection in archival FFPE tissues

To verify our hypothesis, the mutations in exon 9 and exon 20 of *PIK3CA* gene were examined by direct sequencing in 537 primary breast tumor samples including the 24 specimens analyzed by NGS to validate the results of sequencing. Among the succeeded sequenced 494 (92%) samples, *PIK3CA* mutations were determined in 188 (38%) tumors, including 74 mutations in exon 9, 106 mutations in exon 20, and 8 in both exons 9 and 20 (Table 1). The hotspot mutations accounted for 74.6% of total mutations (153/205) in which E542K (p.542), E545K (p.545), and H1047R/L (p.1047) were found in 28, 34, and 91 patients, respectively, with p.1047 ranking the highest (Table 1). Seventeen patients carried two mutations and two of them had H1047R simultaneous with E542K or E545K, which had never been reported before (Table 1). Meanwhile, we characterized 52 non-hotspot

Table 1 *PIK3CA* mutation profiles in exons 9 and 20 in breast cancers (n=494)

Exon	Nucleotide change	Codon change	Effect	Hotspot mutation	Frequency (%)	Number of mutations
9	c.1613A>G	p.Asp538Gly	Missense	No	<1	1
9	c.1615C>T	p.Pro539Ser	Missense	No	<1	1
9	c.1624G>A	p.Glu542Lys	Missense	Yes	13.8	26
9	c.1627A>G	p.Ile543Val	Missense	No	<1	1
9	c.1633G>A	p.Glu545Lys	Missense	Yes	16.5	31
9	c.1633G>C	p.Glu545Gln	Missense	No	<1	1
9	c.1634A>C	p.Glu545Ala	Missense	No	1.6	3
9	c.1634A>G	p.Glu545Gly	Missense	No	<1	2
9	c.1636C>A	p.Gln546Lys	Missense	No	<1	1
9	c.1637A>C	p.Gln546Pro	Missense	No	<1	1
9	c.1637A>G	p.Gln546Arg	Missense	No	1.6	3
9	c.1651C>T	p.Leu551Leu	Silent	No	<1	1
20	c.3120G>A	p.Met1040Ile	Missense	No	<1	2
20	c.3127A>G	p.Met1043Val	Missense	No	<1	2
20	c.3129G>A	p.Met1043Ile	Missense	No	<1	1
20	c.3139C>T	p.His1047Tyr	Missense	No	<1	1
20	c.3140A>G	p.His1047Arg	Missense	Yes	38.3	72
20	c.3140A>T	p.His1047Leu	Missense	Yes	5.3	10
20	c.3145G>C	p.Gly1049Arg	Missense	No	<1	1
20	c.3146G>A	p.Gly1049Asp	Missense	No	<1	1
20	c.3148G>A	p.Gly1050Ser	Missense	No	<1	1
20	c.3152G>A	p.Trp1051*	Nonsense	No	<1	1
20	c.3153G>A	p.Trp1051*	Nonsense	No	<1	1
20	c.3154A>G	p.Thr1052Ala	Missense	No	<1	1
20	c.3155C>T	p.Thr1052Ile	Missense	No	<1	1
20	c.3166G>A	p.Asp1056Asn	Missense	No	<1	1
20	c.3191A>G	p.Gln1064Arg	Missense	No	<1	2
20	c.3194A>T	p.His1065Leu	Missense	No	<1	1
9	c.[1613A>G(+)1633G>A]	p.[Asp538Gly(+)Glu545Lys]	Missense	Yes/No	<1	1
9	c.[1613A>G(+)1633G>C]	p.[Asp538Gly(+)Glu545Gln]	Missense	No	<1	1
9+20	c.[1624G>A(+)3140A>G]	p.[Glu542Lys(+)His1047Arg]	Missense	Yes	<1	1
9+20	c.[1624G>A(+)3146G>A]	p.[Glu542Lys(+)Gly1049Asp]	Missense	Yes/No	<1	1
9+20	c.[1633G>A(+)3140A>G]	p.[Glu545Lys(+)His1047Arg]	Missense	Yes	<1	1
9+20	c.[1633G>A(+)3170G>A]	p.[Glu545Lys(+)Trp1057*]	Nonsense	Yes/No	<1	1
9+20	c.[1636C>A(+)3166A>G]	p.[Gln546Lys(+)Glu1056Asn]	Missense	No	<1	1
9+20	c.[1637A>G(+)3140A>G]	p.[Gln546Arg(+)His1047Arg]	Missense	Yes/No	<1	1
9+20	c.[1637A>G(+)3155C>T]	p.[Gln546Arg(+)Thr1052Ile]	Missense	No	<1	1
9+20	c.[1638G>A(+)3140A>G]	p.[Gln546Gln(+)His1047Arg]	Missense	Yes/No	<1	1
20	c.[3133G>A(+)3152G>A]	p.[Asp1045Asn(+)Trp1051*]	Nonsense	No	<1	1
20	c.[3137C>T(+)3140A>G]	p.[Ala1046Val(+)His1047Arg]	Missense	Yes/No	<1	1
20	c.[3140A>G(+)3145G>A]	p.[His1047Arg(+)Gly1049Ser]	Missense	Yes/No	<1	1
20	c.[3140A>G(+)3146G>A]	p.[His1047Arg(+)Gly1049Asp]	Missense	Yes/No	<1	1
20	c.[3140A>G(+)3156A>T]	p.[His1047Arg(+)Thr1052Thr]	Missense	Yes/No	<1	1
20	c.[3140A>G(+)3166G>A]	p.[His1047Arg(+)Asp1056Asn]	Missense	Yes/No	<1	1
20	c.[3148G>A(+)3153G>A]	p.[Gly1050Ser(+)Trp1051*]	Nonsense	No	<1	1
						Total =188

mutations in which 1 silent and 5 nonsense mutations were observed in 37 patients (Table 1).

In addition, we found 14 new *PIK3CA* mutations in exon 9 and 20 in breast cancers, which have not been reported by COSMIC. Among the newly found mutations, 8 of them were located in exon 9, 4 in exon 20, and the other 2 in both exon 9 and 20 (Table S3). Whether they have any clinical significance needs to be studied further. Thus, these new mutations were not taken for the following analyses in this study.

Association of *PIK3CA* gene mutations with clinicopathological data

As shown in Table 2, *PIK3CA* mutations were positively associated with ER-positive (P=0.016), PR-positive (P=0.007), and low Ki67 labeling index (P=0.001) tumors. Meanwhile, they were negatively correlated with triple-negative breast cancer subtype (P=0.003), but were not associated with age at diagnosis, tumor stage, lymph node status, tumor size, or HER2 status.

We further investigated the relationships between clinicopathological features and *PIK3CA* mutation distributions, including the exon 9/20 and hotspot mutations (p.542/545 and p.1047; Table 2). ER and PR positive were also significantly correlated with mutations in exon 9 and at p.542/545. Besides, the low Ki67 index was found in patients with exon 20 and p.1047 mutations. Meanwhile, triple-negative breast cancer patients had much less p.1047 mutations (P=0.012). In addition, p.1047 mutations were significantly associated with old diagnosis age (≥40 years old; P=0.043).

Compared to breast cancers with *PIK3CA* hotspot mutations, cancers carrying non-hotspot mutations were more likely to belong to triple-negative subtype (P=0.006) and be larger in tumor size (Table 2). When analyzed by mutation burden, cancers with two mutations were more likely to be larger in tumor size compared with cancers harbored only one mutation (Table 2).

Associations of *PIK3CA* gene mutations with prognosis

As shown in Table 2, when we predicted the prognosis of the patients using the NPI method, no significant association was observed between various *PIK3CA* mutation statuses and prognosis.

Furthermore, prognosis analysis was conducted among 303 breast cancer patients with a median follow-up of 35 months. The Cox proportional hazards model and the Kaplan–Meier survival curve were used to evaluate the correlation between PFS rate or OS rate of breast cancer patients and *PIK3CA* mutation statuses.

In the univariate analysis, patients with old prognostic age (P=0.034) and small tumor size (P=0.033) exhibited better PFS, while old prognostic age (P=0.025) also correlated with better OS (Table 3). However, *PIK3CA* mutation frequency was not statistically significantly associated with PFS (HR[95% CI]=1.257[0.732–2.160], P=0.407), OS (HR[95% CI]=1.946[0.987–3.837], P=0.055), as well as their exon 9, exon 20, and hotspot mutations (Table 3).

When examined by Kaplan–Meier estimate and log-rank test, the PFS of total patients with *PIK3CA* mutations was almost the same as wild-type patients, while the OS was significantly better in the total patients with *PIK3CA* mutations (Figure 2). However, there were no differences in PFS/OS between mutation and wild-type groups when examined by exon 9, exon 20, and hotspot mutations p.542/545 and p.1047 (Figures S1 and S2). Besides, *PIK3CA* mutations significantly improved the OS of the patients with old diagnosis age, low Ki67 labeling index, or luminal/HER2-enriched subtypes (Figure 3). Also as better prognostic effectors, exon 20 mutations as well as the hotspot p.1047 mutations were significantly associated with the PFS of the patients in HER2-negative or low Ki67 labeling index subgroups (Figure 4) and the OS of the patients diagnosed as luminal/HER2-enriched subtypes (Figure 3). In contrast, exon 9 mutations and its hotspot p.542/545 mutations were found in the patients with worse PFS, who belonged to PR-positive or lymph node-negative subgroups (Figure 4).

When performing the univariate Cox analysis according to different clinicopathological parameters, a significant difference in PFS was observed between prognosis and exon 9 as well as p.542/545 hotspot mutations in PR-positive or lymph node-negative subgroups, exon 20 in HER2-negative or low Ki67 labeling index subgroups (Table 4). In OS, a significantly better prognosis was found in total *PIK3CA* and exon 20 mutations patients with luminal/HER2-enriched subtypes, while total *PIK3CA* mutations patients with old diagnostic age had a better OS as well (Table 4). These results were partially in accordance with the Kaplan–Meier analysis. Besides, no significance was detected between prognosis and *PIK3CA* mutation distribution/bias/burden under multiple other clinical, pathological, and molecular subtypes (Table S4).

Discussion

To study the clinicopathological and prognostic values of *PIK3CA* variants in the breast cancer patients from Central China, 494 patients were investigated, and new insight into the complexity of *PIK3CA* mutations was provided in this research. In general, the mutation frequency (38%) in this study is relatively high as most investigations reported ~30% mutation rate using the similar detection method (Table 5).[21,24,26,35–42] Interestingly, the frequency rates of *PIK3CA* mutations fluctuated among the studies which had been done by different groups from distinct areas of China (Table S5).[43–54] This might be partially due to the sensitivity of assay methods. However, considering the controversial

Table 2 Association between *PIK3CA* mutations and standard clinical, pathological, and biological features of breast cancer

Parameters	Category	n	*PIK3CA*		Exon 9		Exon 20		p.542/545		p.1047		*PIK3CA* mutation			*PIK3CA* mutation burdens		
			Mut	*P*-value	Mut	*P*-value	Mut	*P*-value	Mut	*P*-value	Mut	*P*-value	Hotspot	Non-hotspot	*P*-value	1 Mut	2 Muts	*P*-value
Total		494	188 (38%)		82 (17%)		114 (23%)		62 (13%)		91 (18%)		153 (75%)	52 (25%)		171 (91%)	17 (9%)	
Age (years)	<40	91	28 (31%)	0.106	15 (16%)	0.974	15 (16%)	0.098	11 (12%)	0.883	10 (11%)	**0.043**	21 (66%)	11 (34%)	0.202	24 (86%)	4 (14%)	0.294
	≥ 40	403	160 (40%)		67 (17%)		99 (25%)		51 (13%)		81 (20%)		132 (76%)	41 (24%)		147 (92%)	13 (8%)	
Tumor stage	I	12	4 (33%)	0.216	0 (0%)	0.191	4 (33%)	0.413	0 (0%)	0.280	3 (25%)	0.580	3 (75%)	1 (25%)	1.000	4 (100%)	0 (0%)	0.776
	II	250	105 (42%)		46 (18%)		63 (25%)		35 (14%)		51 (20%)		86 (75%)	28 (25%)		96 (91%)	9 (9%)	
	III	205	70 (34%)		31 (15%)		43 (21%)		23 (11%)		35 (17%)		58 (75%)	19 (25%)		63 (90%)	7 (10%)	
	Unknown	27	9		5		4		4		2		6	4		8	1	
Lymph node status	0	229	86 (38%)	0.696	34 (15%)	0.543	54 (24%)	0.930	25 (11%)	0.637	41 (18%)	0.780	66 (70%)	28 (30%)	0.199	78 (91%)	8 (9%)	0.391
	1–3	108	45 (42%)		21 (19%)		26 (24%)		15 (14%)		19 (18%)		34 (72%)	13 (28%)		43 (96%)	2 (4%)	
	>3	131	48 (37%)		23 (18%)		29 (22%)		18 (14%)		27 (21%)		45 (83%)	9 (17%)		42 (88%)	6 (12%)	
	Unknown	26	9		4		5		4		4		8	2		8	1	
Tumor size (cm)	<2	124	42 (34%)	0.523	19 (15%)	0.401	24 (19%)	0.209	14 (11%)	0.179	22 (18%)	0.917	36 (84%)	7 (16%)	0.071	41 (98%)	1 (2%)	0.074
	2–5	315	125 (40%)		57 (18%)		72 (23%)		45 (14%)		58 (18%)		103 (75%)	34 (25%)		113 (90%)	12 (10%)	
	≥5	54	20 (37%)		6 (11%)		17 (31%)		3 (6%)		11 (20%)		14 (58%)	10 (42%)		16 (80%)	4 (20%)	
	Unknown	1	1		0		1		0		0		0	1		1	0	
Molecular subtypes	Luminal A	69	31 (45%)	**0.003**	15 (21%)	0.069	17 (25%)	0.114	9 (13%)	0.163	13 (19%)	**0.012**	22 (67%)	11 (33%)	**0.006**	29 (94%)	2 (6%)	0.498
	Luminal B	300	120 (40%)		54 (18%)		72 (24%)		43 (14%)		58 (19%)		101 (78%)	29 (22%)		110 (92%)	10 (8%)	
	HER2+	66	27 (41%)		10 (15%)		18 (27%)		8 (12%)		17 (26%)		25 (86%)	4 (14%)		25 (93%)	2 (7%)	
	Triple Negative	57	9 (16%)		3 (5%)		6 (11%)		2 (4%)		2 (4%)		4 (36%)	7 (64%)		7 (78%)	2 (22%)	
	Unknown	2	1		0		1		0		1		1	1		0	1	
ER	Positive	357	148 (41%)	**0.014**	68 (19%)	**0.025**	87 (24%)	0.260	57 (16%)	**0.049**	70 (20%)	0.334	127 (79%)	33 (21%)	0.708	136 (92%)	12 (8%)	0.670
	Negative	133	39 (29%)		14 (11%)		26 (20%)		12 (9%)		21 (16%)		33 (77%)	10 (23%)		35 (90%)	4 (10%)	
	Unknown	4	1		0		1		0		1		1	1		0	1	
PR	Positive	319	134 (42%)	**0.015**	61 (19%)	**0.050**	80 (25%)	0.139	53 (17%)	0.026	64 (20%)	0.235	117 (81%)	28 (19%)	0.302	123 (92%)	11 (8%)	0.787
	Negative	172	53 (31%)		21 (12%)		33 (19%)		16 (9%)		27 (16%)		43 (74%)	15 (26%)		48 (91%)	5 (9%)	
	Unknown	3	1		0		1		0		1		1	1		0	1	
HER2	Positive	172	69 (40%)	0.519	26 (15%)	0.507	47 (27%)	0.111	22 (13%)	0.896	36 (21%)	0.303	58 (76%)	18 (24%)	0.691	62 (90%)	7 (10%)	0.564
	Negative	315	117 (37%)		55 (18%)		66 (21%)		39 (12%)		54 (17%)		93 (74%)	33 (26%)		108 (92%)	9 (8%)	
	Unknown	7	2		1		1		1		1		2	1		1	1	
Ki67 (%)	≤30	250	114 (46%)	**0.001**	46 (18%)	0.424	72 (29%)	**0.002**	33 (13%)	0.847	62 (25%)	**0.000**	95 (80%)	27 (22%)	0.189	106 (93%)	8 (7%)	0.317
	>30	230	71 (31%)		36 (16%)		39 (17%)		29 (13%)		26 (11%)		55 (70%)	24 (30%)		63 (89%)	8 (11%)	
	Unknown	14	3		0		3		0		3		3	1		2	1	
NPI	Good (2–3.4)	82	35 (43%)	0.671	15 (18%)	0.301	21 (26%)	0.662	11 (13%)	0.501	17 (21%)	0.788	28 (78%)	8 (22%)	0.883	34 (97%)	1 (3%)	0.392
	Moderate (3.4–5.4)	213	79 (37%)		29 (14%)		53 (25%)		22 (10%)		42 (20%)		64 (74%)	23 (26%)		71 (90%)	8 (10%)	
	Poor (>5.4)	155	59 (38%)		30 (19%)		33 (21%)		22 (14%)		27 (17%)		49 (75%)	16 (25%)		53 (90%)	6 (10%)	
	Unknown	44	15		8		7		7		5		12	5		13	2	

Note: *P*<0.05 was considered statistically significant and those values are shown in bold.

Abbreviations: ER, estrogen receptor; HER2, human epidermal growth factor receptor 2; Mut, mutant; NPI, Nottingham Prognostic Index; PR, progesterone receptor.

Table 3 Univariate Cox analysis of the correlation between clinicopathological parameters and progression-free/overall survival of breast cancer patients

Variables	PFS		OS	
	HR (95% CI)	*P*-value	HR (95% CI)	*P*-value
Age (years)				
<40 vs ≥40	1.805 (1.044–3.121)	**0.034**	2.050 (1.092–3.848)	**0.025**
Tumor stage				
I and II vs III	1.692 (0.976–2.935)	0.061	1.465 (0.769–2.792)	0.245
Lymph node status				
Positive vs negative	0.764 (0.452–1.290)	0.313	0.944 (0.521–1.711)	0.850
Tumor size				
<5 cm vs ≥5 cm	0.476 (0.240–0.943)	**0.033**	0.493 (0.229–1.062)	0.071
Molecular subtypes				
Luminal and HER2+ vs triple negative	1.264 (0.572–2.791)	0.563	0.939 (0.417–2.110)	0.878
ER				
Positive vs negative	1.162 (0.685–1.971)	0.579	1.114 (0.600–2.068)	0.732
PR				
Positive vs negative	1.469 (0.887–2.431)	0.135	1.403 (0.782–2.518)	0.257
HER2				
Positive vs negative	0.735 (0.433–1.246)	0.253	0.737 (0.396–1.371)	0.335
Ki67				
≤30% vs >30%	0.980 (0.583–1.646)	0.939	0.725 (0.389–1.349)	0.309
***PIK3CA* mutational status**				
PIK3CA mutation vs wild-type	1.257 (0.732–2.160)	0.407	1.946 (0.987–3.837)	0.055
Exon 9 mutation vs wild-type	0.794 (0.389–1.621)	0.527	1.444 (0.515–4.050)	0.485
Exon 20 mutation vs wild-type	1.696 (0.883–3.259)	0.113	1.950 (0.907–4.194)	0.087
p.542/545 mutation vs wild-type	0.731 (0.346–1.547)	0.413	1.646 (0.508–5.329)	0.406
p.1047 mutation vs wild-type	1.662 (0.819–3.371)	0.159	2.2025 (0.857–4.783)	0.108

Note: *P*<0.05 was considered statistically significant and those values are shown in bold.
Abbreviations: ER, estrogen receptor; HER2, human epidermal growth factor receptor 2; OS, overall survival; PFS, progression-free survival; PR, progesterone receptor.

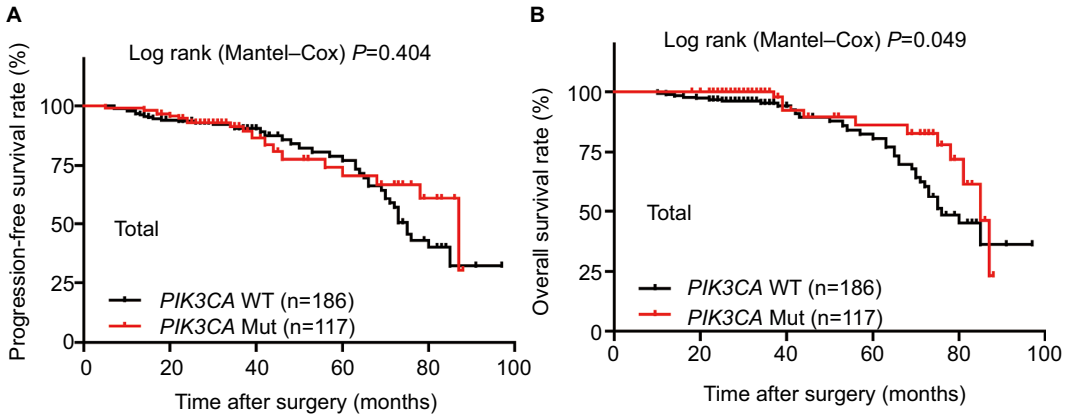

Figure 2 Kaplan–Meier survival curves according to *PIK3CA* genotype for (**A**) progression-free survival and (**B**) overall survival of the total patients.
Abbreviations: Mut, mutation; WT, wild-type.

results from the researches around the world as well as environmental factors and lifestyles playing roles in breast cancer, we hypothesized that *PIK3CA* mutations and its associated factors might show diversity roles among the populations from different regions.

Then, we identified a significant association of *PIK3CA* mutations with clinicopathological and molecular characteristics, such as luminal/HER2-enriched subtypes, ER-positive, PR-positive, and low Ki67 labeling index which were partly consistent with the literature.[26,35,36,40,41,44,45,47] When we separately analyzed the mutations in exon 9 and exon 20, their differences on the relationships with the clinicopathological characteristics were identified and should be considered separately when used for disease monitoring, therapeutic

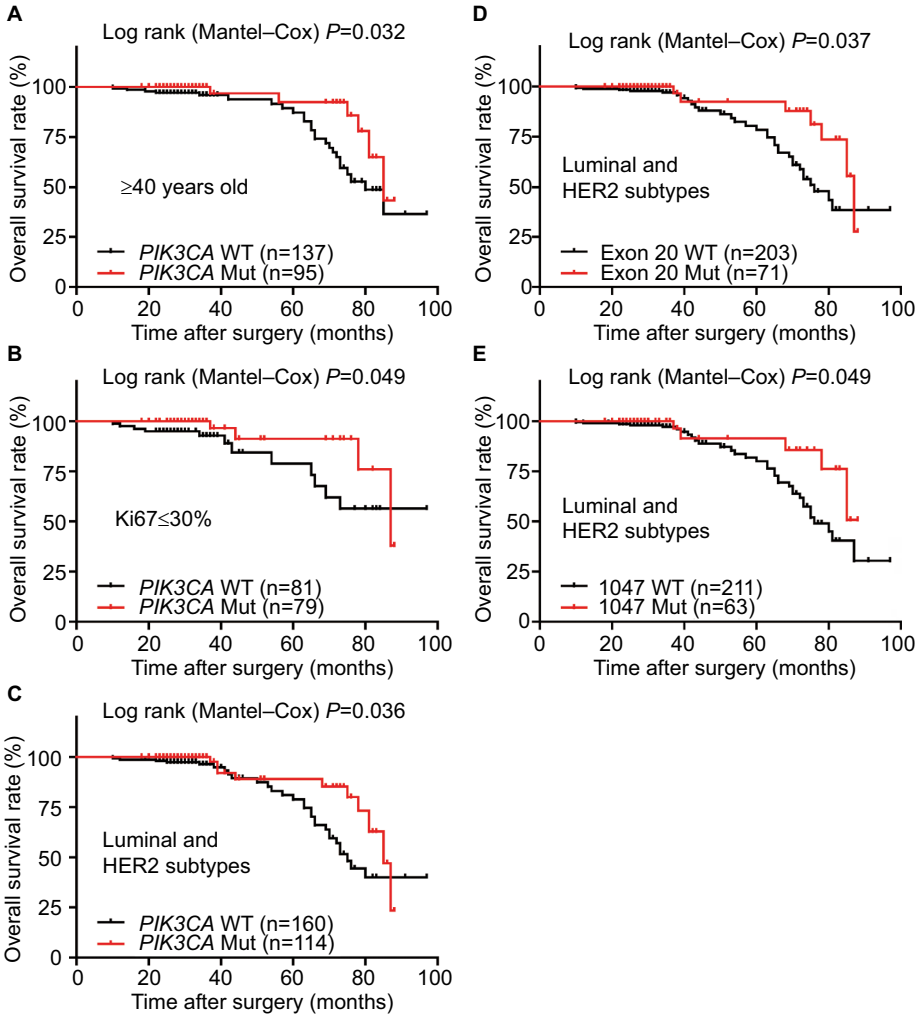

Figure 3 Overall Kaplan–Meier survival curves.
Note: (A,B) OS rates of the *PIK3CA* mutations in ≥40 years old (**A**) and Ki67 labeling index ≤30% (**B**) subgroups; (**C**–**E**) OS rates of the luminal and HER2-enriched subtypes patients with *PIK3CA* (**C**), exon 20 (**D**), or p.1047 (**E**) mutations.
Abbreviations: HER2, human epidermal growth factor receptor 2; Mut, mutation; OS, overall survival; WT, wild-type.

effect evaluation, and prognosis prediction. Besides, the point mutation p.1047, non-hotspot mutations, and more mutation burdens related to specific clinical and biological features of breast cancer might play particular roles and need to be investigated in future.

Numerous investigators reported that *PIK3CA* mutations are associated with prognosis.[24,26,39–41,43,44] However, this association varies and even contradict among studies. Some of them showed better prognosis of the patients with *PIK3CA* mutations,[24,26,40] and others believed worse outcome,[39,43,44,50] while many researchers did not find any prognostic significance.[35,36,38,49] In our study, interesting outcomes were explored when we tested the prognostic value of each *PIK3CA* mutation status in the subgroups separated according to different clinicopathological parameters. Firstly, total *PIK3CA* mutations exhibited disparate roles between FPS and OS in subgroups (Figures 2 and 3). Secondly, both exon 9 and exon 20 mutations correlated with FPS, but in diverse subgroups (Figure 4). Thirdly, only the effect of exon 20 mutations on the OS was identified (Figure 3D). Furthermore, exon 9 and exon 20 mutations revealed completely converse roles in the prognosis (Figures 3D and 4). Moreover, the hotspot mutations were in perfect accord with their exons (Figures 3D, E and 4). These results demonstrated that the variant status of *PIK3CA* mutations played different roles in the prognosis of breast cancer patients in our area.

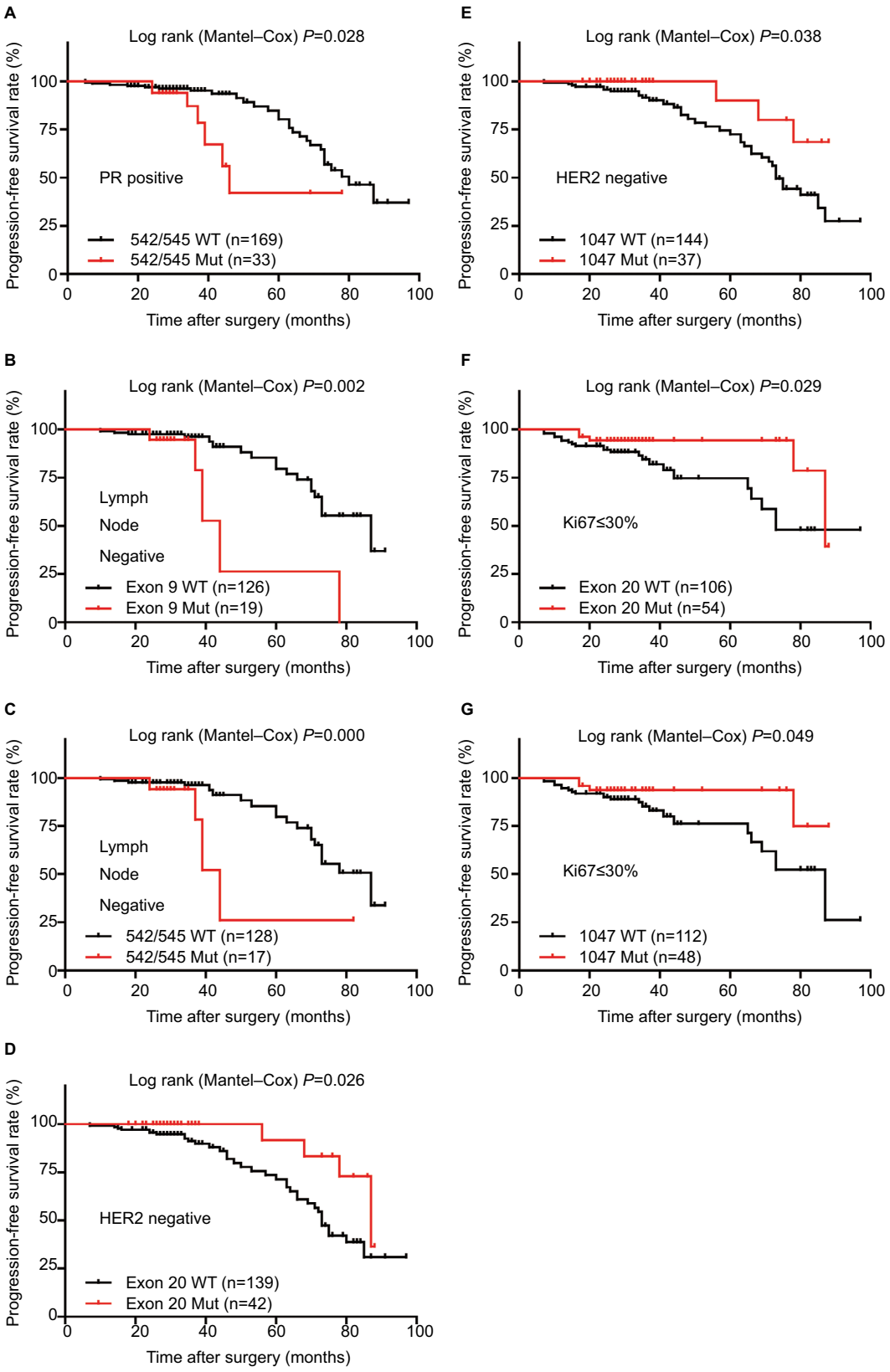

Figure 4 Progression-free Kaplan–Meier survival curves.

Note: (**A**) PFS rates of the PR-positive patients with p.542/545 mutations; (**B,C**) PFS rates of the lymph node-negative patients with exon 9 (**B**) or p.542/545 (**C**) mutations; (**D,E**) PFS rates of the HER2-negative patients with exon 20 (**D**) or p.1047 (**E**) mutations; (**F,G**) PFS rates of the Ki67 labeling index ≤30% patients with exon 20 (**F**) or p.1047 (**G**) mutations.

Abbreviations: HER2, human epidermal growth factor receptor 2; Mut, mutation; PFS, progression-free survival; PR, progesterone receptor; WT, wild-type.

Table 4 Univariate Cox analysis of the correlation between *PIK3CA* mutation status and progression-free/overall survival according to different clinicopathological parameters

Variables	PFS		OS	
	HR (95% CI)	*P*-value	HR (95% CI)	*P*-value
≥40 years old				
PIK3CA mutation vs wild-type	1.632 (0.821–3.247)	0.163	2.545 (1.042–6.215)	**0.040**
PR positive				
p.542/545 mutation vs wild-type	0.397 (0.169–0.932)	**0.034**	0.980 (0.293–3.282)	0.974
HER2 negative				
Exon 20 mutation vs wild-type	3.039 (1.078–8.573)	**0.036**	2.156 (0.750–6.198)	0.154
Ki67 labeling index ≤30%				
Exon 20 mutation vs wild-type	2.838 (1.065–7.565)	**0.037**	3.099 (0.869–11.047)	0.081
Lymph node negative				
Exon 9 mutation vs wild-type	0.236 (0.085–0.655)	**0.006**	0.444 (0.127–1.545)	0.202
p.542/545 mutation vs wild-type	0.107 (0.029–0.403)	**0.001**	0.401 (0.090–1.790)	0.231
Luminal and HER2 subtypes				
PIK3CA mutation vs wild–type	1.323 (0.752–2.327)	0.332	2.125 (1.032–4.375)	**0.041**
Exon 20 mutation vs wild-type	1.969 (0.988–3.923)	0.054	2.337 (1.024–5.331)	**0.044**

Note: *P*<0.05 was considered statistically significant and those values are shown in bold.
Abbreviations: HER2, human epidermal growth factor receptor 2; PFS, progression-free survival; PR, progesterone receptor; OS, overall survival.

Table 5 Comparison of association between *PIK3CA* mutations and various clinicopathological features in different studies

Authors	Year of publication	Country	No. of patients	*PIK3CA* mutation	Sample type	Methods	Association between *PIK3CA* mutations and clinicopathological characteristics
Arsenic et al[21]	2014	Germany	241	15.8%	FTS	DS	H1047R mutation: worse overall survival
Barbareschi et al[24]	2007	Italy	163	27.6%	FTS	SSCP + DS	Exon 20 mutations: prolonged overall and disease-free survival; exon 9 mutations: poor prognosis for disease-free survival and overall survival
Bozhanov et al[35]	2010	Bulgaria	145	31.3%	FTS	SSCP + DS	*PIK3CA* mutations: PR positive
Cizkova et al[26]	2012	France	452	33.4%	FTS	DS	*PIK3CA* mutations: low histopathological grade, small macroscopic tumor size, ER positive, PR positive, HER2 negative, favorable metastasis-free survival
Kalinsky et al[36]	2009	USA	590	33%	FFPE	MA + DS	*PIK3CA* mutations: ER positive, PR positive, HER2 negative, low-grade tumor; exon 9 mutations: older age; exon 20 mutations: node negative
Liang et al[37]	2006	Singapore	80	39%	FFPE	DS	Exon 20 mutations: older age, early stage
López-Knowles et al[38]	2010	Australia	168	7%	FFPE	DS	No significance
Mangone et al[39]	2012	Brazil	86	27%	FTS	SSCP + DS	Exon 20 mutations: poor overall survival and disease-free survival
Maruyama et al[40]	2007	Japan	188	24.47%	FTS	DS	*PIK3CA* mutations: ER positive, favorable prognosis
Pérez-Tenorio et al[41]	2007	Sweden	270	24%	FTS	SSCP + DS	*PIK3CA* mutations: ER positive, small tumor size, HER2 negative, longer local recurrence-free survival
Azizi Tabesh et al[42]	2016	Iran	80	45%	FTS	DS	*PIK3CA* mutations: low grade; Exon 20 mutations: PR positive
Current study	2018	China	494	38.06%	FFPE	DS	*PIK3CA* mutations: luminal and HER2 positive, ER positive, PR positive, low Ki67 index, better overall survival; exon 9 mutations: worse progressive-free survival; exon 20 mutations: better progressive-free survival and overall survival; H1047 mutations: older age, better progressive-free survival and overall survival; non-hotspot mutations: larger tumor size

Abbreviations: DS, direct sequencing; ER, estrogen receptor; FFPE, formalin-fixed, paraffin-embedded tissue samples; FTS, frozen tissue samples; HER2, human epidermal growth factor receptor 2; MA, MassARRAY; PR, progesterone receptor; SSCP, single-strand conformation polymorphism.

In addition, when checking our samples, we realized that 60% of tumors in our study belonged to luminal B molecular subtype, which was extremely higher than the ratio in the other studies (~30%). This phenomenon also demonstrated that breast cancer patients in our area might have some specific preferences in genetic and clinicopathological features. However, the reasons and mechanisms need to be elucidated in future.

Limitations

This study still has some limitations. Firstly, all the samples were from a single center with a relatively small sample size. Although we identified some rules in the clinicopathological features, prognostic relevance, and *PIK3CA* mutation preferences, the sample size in the subgroups (stage I tumors, tumors with two mutations, and the relapsed patients) was quite small which made the results not that solid. Secondly, the follow-up times for most patients were too short. As >80% of breast cancer patients survive for >5 years after diagnosis, longer follow-up time is needed. Moreover, the effect and association of adjuvant systemic therapy with *PIK3CA* mutation status were not evaluated in this study, which might influence the progression-free/overall survival rate. Furthermore, the oncogenetic mutations in other exons were not examined, which might contribute to the prognosis of the patients.

Conclusion

PIK3CA mutations were detected using Sanger sequencing combined with targeted NGS in 38% of breast cancer patients from a single hospital in Central China. Different from the other studies, 60% of breast cancer patients were diagnosed with luminal B tumors. *PIK3CA* mutations were associated with ER-positive, PR-positive, low Ki67 labeling index, and luminal/HER2-enriched subtypes, while exon 9, exon 20, hotspot mutations, and mutation burdens made distinct contributions. In addition, p.1047 mutations were significantly associated with older diagnosis age. Significant heterogeneity was identified in the univariable effect of *PIK3CA* mutation status on FPS and OS. *PIK3CA* mutations patients had a better OS, which was also showed in the older diagnostic age, PR-negative, low Ki67 labeling index, and luminal/HER2-enriched subgroups. As better prognostic markers, exon 20 and p.1047 hotspot mutations significantly persisted in the HER2-negative and low Ki67 labeling index subgroups (analyzed by FPS) as well as luminal/HER2-enriched subgroup (analyzed by OS). In contrast, exon 9 and p.542/545 hotspot mutations exhibited worse prognostic factors in PR-positive and lymph node-negative subgroups when assayed using FPS. Therefore, these results demonstrated that the mutation frequency, distribution, bias, and burden of *PIK3CA* were related to different clincopathological characteristics, prognosis, and might play different roles in breast cancer from Central China. These differences and relationships should be deeply studied and taken into consideration during disease management.

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Disclosure

The authors report no conflicts of interest in this work.

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Supplementary materials

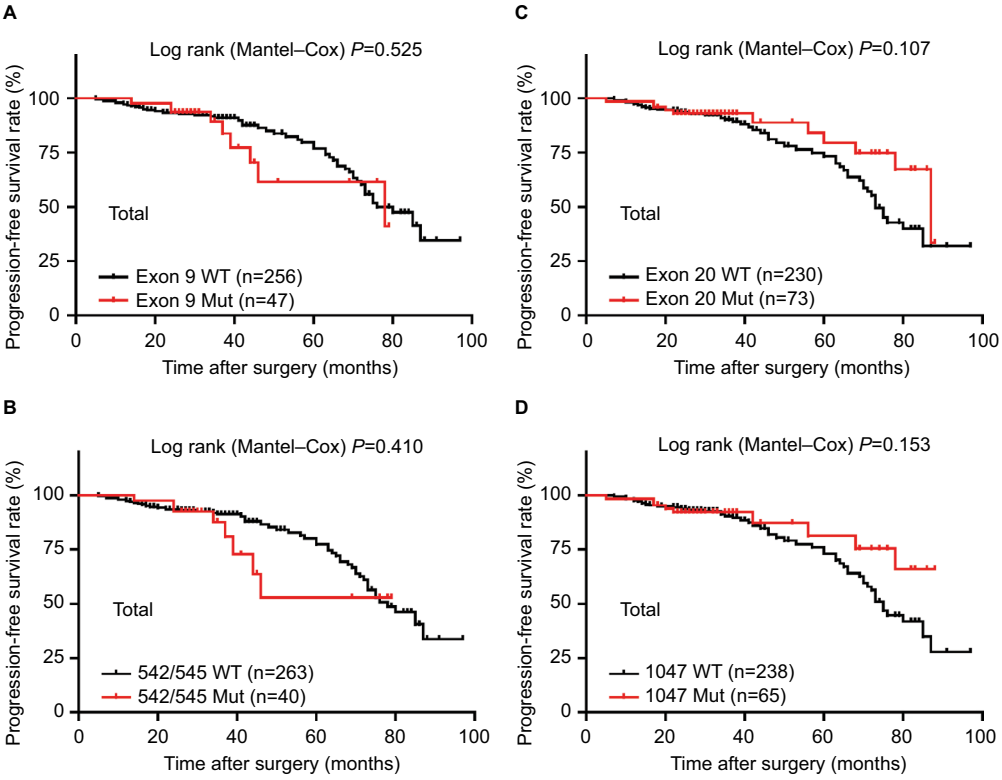

Figure S1 Progression-free Kaplan–Meier survival curves of the total patients.
Note: (**A**–**D**) PFS rate of total patients with exon 9 (**A**), p.542/545 (**B**), exon 20 (**C**), or p.1047 (**D**) mutations.
Abbreviations: Mut, mutation; PFS, progression-free survival; WT, wild-type.

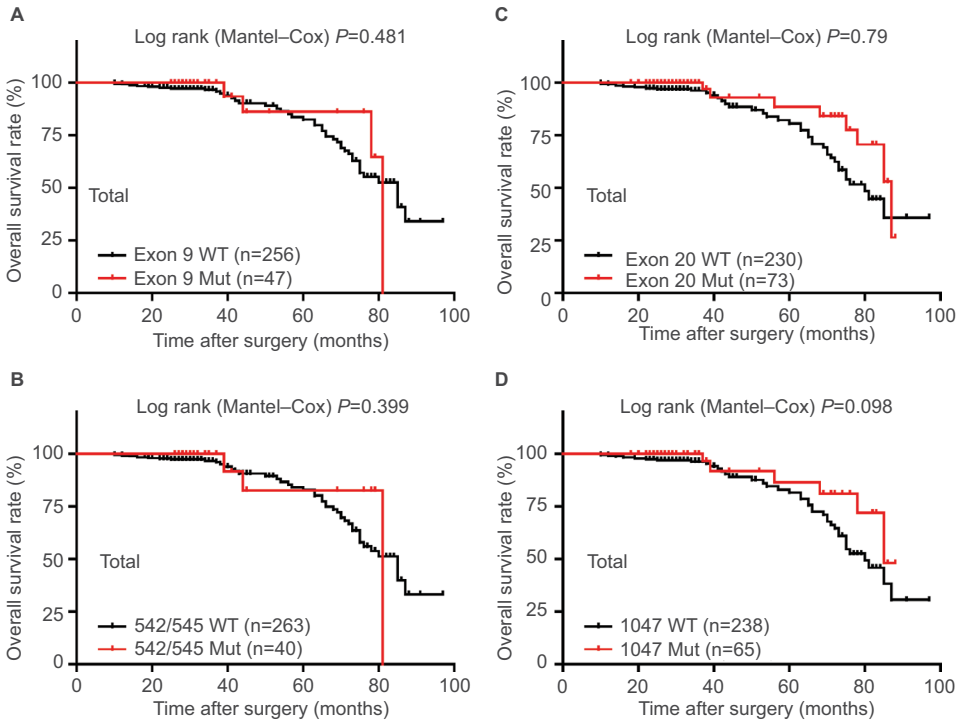

Figure S2 Overall Kaplan–Meier survival curves of the total patients.
Note: (**A**–**D**) OS rate of total patients with exon 9 (**A**), p.542/545 (**B**), exon 20 (**C**), or p.1047 (**D**) mutations.
Abbreviations: Mut, mutation; OS, overall survival; WT, wild-type.

Table S1 Clinical, pathological, and biological features of breast cancer patients

Parameters	Category	Number of patients	Percentage	Note
Total		494	100.00	Eligible samples for analysis in 537 samples
Age (years)	<40	91	18.40	Age range: 25–89 years old
	≥40	403	81.58	Median age: 48 years old
Tumor stage	I	12	2.43	
	II	250	50.61	
	III	205	41.50	
	Unknown	27	5.47	
Lymph node status	0	229	46.36	
	1–3	108	21.86	
	>3	131	26.52	
	Unknown	26	5.26	
Tumor size (cm)	<2	124	25.10	Tumor size range: 0.3–12 cm
	2–5	315	63.77	Median size: 2.6 cm
	≥5	54	10.93	
	Unknown	1	0.20	
Molecular subtypes	Luminal A	69	13.97	
	Luminal B	300	60.73	
	HER2+	66	13.36	
	Triple negative	57	11.54	
	Unknown	2	0.40	
ER	Positive	357	72.27	
	Negative	133	26.92	
	Unknown	4	0.81	
PR	Positive	319	64.57	
	Negative	172	34.82	
	Unknown	3	0.61	
HER2	Positive	172	34.82	
	Negative	315	63.77	
	Unknown	7	1.42	
Ki67 (%)	≤30	250	50.61	
	>30	230	46.56	
	Unknown	14	2.83	
Follow-up data collected	Total	303	61.34	Follow-up period: 5–97 months
	Relapsed or second tumor	28	9.24[a]	Median time: 35 months
	Died	46	15.18[a]	

Note: [a]Percentage of the total follow-up data collected samples.
Abbreviations: ER, estrogen receptor; HER2, human epidermal growth factor receptor 2; PR, progesterone receptor.

Table S2 General clinical and pathological features of breast cancers used for targeted sequencing (n=24)

Patient ID	*PIK3CA* mutation	Age (years)	Tumor stage	Lymph node status	Tumor size (cm)	Molecular subtype	ER	PR	HER2	Ki67	PFS (Month)	Note
R160066	WT	39	III	8	3	Luminal B	90%	90%	2+	20%	24	
R160069	WT	30	III	12	3	HER2+	Negative	Negative	3+	60%	37	
R160070	WT	31	III	0	6	Luminal B	30%	10%	Negative	50%	Unknown	
R160071	WT	32	II–III	2	2	Luminal B	70%	40%	2+	30%	40	
R160080	WT	35	II	4	3	Luminal B	50%	50%	1+	20%	Unknown	
R160082	WT	35	Unknown	23	4.5	Luminal B	90%	30%	1+	Unknown	43	
R160088	WT	37	II–III	4	1.6	Luminal B	80%	15%	3+	20%	44	
R160090	E542K	37	II–III	1	2.2	Luminal B	95%	5%	Negative	60%	35	Relapsed
R160093	WT	39	II–III	0	3.5	Luminal B	95%	95%	2+	15%	40	
R160104	WT	43	II	2	2	Luminal B	100%	100%	Negative	30%	22	
R160107	E545K	43	Unknown	24	3.5	Luminal B	90%	30%	1+	30%	27	
R160110	WT	43	II–III	0	2.2	Luminal B	90%	90%	3+	40%	29	
R160111	WT	43	III	0	1	Luminal B	40%	90%	3+	70%	23	
R160114	WT	44	II	0	1.5	Luminal B	90%	90%	1+	60%	23	
R160123	E545K	45	II	2	2.3	Luminal B	95%	95%	Negative	35%	28	
R160127	WT	45	II	0	1	Luminal B	80%	70%	Negative	25%	21	
R160130	WT	45	III	1	2.5	Triple negative	Negative	Negative	1+	80%	23	
R160133	E542K	46	II	0	4.2	Luminal B	95%	95%	Negative	25%	Unknown	
R160138	WT	46	III	0	4	Triple negative	Negative	Negative	Negative	80%	22	
R160145	E542K	47	II	0	1.8	Luminal B	50%	20%	Negative	75%	26	
R160158	WT	48	III	6	1.3	Luminal B	30%	Negative	Negative	60%	28	
R160161	E545K	49	Unknown	10	8	Luminal B	50%	50%	Negative	20%	36	Relapsed
R160164	H1047R	50	II–III	0	2	Luminal B	3%	Negative	Negative	60%	27	
R160169	H1047R	51	II	12	2	Luminal A	90%	30%	1+	8%	30	

Abbreviations: ER, estrogen receptor; HER2, human epidermal growth factor receptor 2; PFS, progression-free survival; PR, progesterone receptor; WT, wild-type.

Table S3 New *PIK3CA* mutations in exons 9 and 20 in breast cancers (n=494)

Exon	Nucleotide change	Codon change	New mutation effect	Number of mutations
9	c.1618C>G	p.Leu540Val	Missense	1
9	c.1621T>C	p.Ser541Pro	Missense	1
9	c.1628T>C	p.Ile543Thr	Missense	1
9	c.1628T>G	p.Ile543Ser	Missense	1
9	c.1629C>G	p.Ile543Met	Missense	2
9	c.1655G>A	p.Trp552*	Nonsense	1
20	c.3139_3140CA>TG	p.His1047Cys	Missense	1
20	c.3206G>A	p.*1069*	Silent	1
9	c.[1621T>C(+)1644delA]	p.[Ser541Pro(+)Lys548fs*10]	Frameshift	1
9+20	c.[1651C>T(+)3117C>T]	p.[Leu551Leu(+)Phe1039Phe]	Silent	1
20	c.[3120G>A(+)3201G>A]	p.[Met1040Ile(+)Leu1067Leu]	Silent	1
9+20	c.[1644A>G(+)3140A>G(+)3178C>T]	p.[Lys548Lys(+)His1047Arg(+)His1060Tyr]	Missense	1
20	c.[3117C>T(+)3140A>G(+)3145G>A]	p.[Phe1039Phe(+)His1047Arg(+)Gly1049Ser]	Silent	1

Table S4 Univariate Cox analysis of the correlation between *PIK3CA* mutation status and progression-free/overall survival according to different clinicopathological parameters

Variables	PFS		OS	
	HR (95% CI)	*P*-value	HR (95% CI)	*P*-value
<40 years old				
PIK3CA mutation vs wild-type	0.746 (0.298–10.866)	0.531	1.365 (0.463–4.022)	0.572
Exon 9 mutation vs wild-type	0.554 (0.173–1.780)	0.322	1.345 (0.298–6.077)	0.700
Exon 20 mutation vs wild-type	1.050 (0.336–3.282)	0.934	1.238 (0.339–4.519)	0.747
p.542/545 mutation vs wild-type	0.382 (0.117–1.247)	0.111	0.995 (0.221–4.470)	0.995
p.1047 mutation vs wild-type	0.894 (0.256–3.117)	0.860	1.133 (0.252–5.092)	0.871
≥40 years old				
PIK3CA mutation vs wild-type	1.632 (0.821–3.247)	0.163	2.545 (1.042–6.215)	**0.040**
Exon 9 mutation vs wild-type	1.029 (0.403–2.628)	0.953	1.858 (0.440–7.837)	0.399
Exon 20 mutation vs wild-type	1.977 (0.879–4.446)	0.099	2.283 (0.874–5.962)	0.092
p.542/545 mutation vs wild-type	1.068 (0.380–3.003)	0.901	3.175 (0.431–23.373)	0.257
p.1047 mutation vs wild-type	1.942 (0.819–4.607)	0.132	2.412 (0.840–6.928)	0.102
ER negative				
PIK3CA mutation vs wild-type	1.747 (0.589–5.181)	0.314	5.336 (0.699–40.709)	0.106
Exon 9 mutation vs wild-type	1.507 (0.202–11.232)	0.689	–	–
Exon 20 mutation vs wild-type	1.711 (0.504–5.810)	0.389	3.824 (0.500–29.247)	0.196
p.542/545 mutation vs wild-type	1.507 (0.202–11.232)	0.689	–	–
p.1047 mutation vs wild-type	1.497 (0.440–5.092)	0.518	3.312 (0.433–25.325)	0.248
ER positive				
PIK3CA mutation vs wild-type	1.060 (0.555–2.025)	0.860	1.583 (0.736–3.403)	0.240
Exon 9 mutation vs wild-type	0.665 (0.303–1.459)	0.309	1.185 (0.410–3.425)	0.754
Exon 20 mutation vs wild-type	1.707 (0.779–3.741)	0.182	1.714 (0.727–4.041)	0.219
p.542/545 mutation vs wild-type	0.579 (0.253–1.324)	0.195	1.295 (0.390–4.295)	0.673
p.1047 mutation vs wild-type	1.785 (0.744–4.279)	0.194	1.801 (0.684–4.738)	0.233
PR negative				
PIK3CA mutation vs wild-type	1.522 (0.657–3.526)	0.327	3.198 (0.953–10.729)	0.060
Exon 9 mutation vs wild-type	1.888 (0.451–7.908)	0.384	–	–
Exon 20 mutation vs wild-type	1.347 (0.517–3.510)	0.542	2.099 (0.623–7.070)	0.231
p.542/545 mutation vs wild-type	1.665 (0.397–6.975)	0.486	–	–
p.1047 mutation vs wild-type	1.159 (0.444–3.023)	0.763	1.860 (0.552–6.271)	0.317
PR positive				
PIK3CA mutation vs wild-type	0.983 (0.468–2.066)	0.964	1.396 (0.582–3.353)	0.455
Exon 9 mutation vs wild-type	0.406 (0.170–0.973)	**0.043**	0.733 (0.246–2.187)	0.578
Exon 20 mutation vs wild-type	2.014 (0.811–4.999)	0.131	1.880 (0.686–5.1479)	0.220
p.542/545 mutation vs wild-type	0.340 (0.134–0.863)	**0.023**	0.786 (0.231–2.674)	0.700
p.1047 mutation vs wild-type	2.317 (0.801–6.704)	0.121	2.258 (0.664–7.677)	0.192
HER2 negative				
PIK3CA mutation vs wild-type	1.496 (0.727–3.076)	0.274	2.168 (0.885–5.314)	0.091
Exon 9 mutation vs wild-type	0.507 (0.221–1.166)	0.110	1.238 (0.373–4.112)	0.728
Exon 20 mutation vs wild-type	3.039 (1.078–8.573)	**0.036**	2.156 (0.750–6.198)	0.154
p.542/545 mutation vs wild-type	0.452 (0.187–1.096)	0.079	1.546 (0.366–6.526)	0.553
p.1047 mutation vs wild-type	3.225 (0.991–10.488)	0.052	2.262 (0.685–7.472)	0.180
HER2 positive				
PIK3CA mutation vs wild-type	0.948 (0.410–2.193)	0.900	1.983 (0.630–6.239)	0.242
Exon 9 mutation vs wild-type	1.709 (0.400–7.306)	0.470	1.964 (0.257–15.004)	0.515
Exon 20 mutation vs wild-type	0.903 (0.370–2.205)	0.823	2.248 (0.627–8.065)	0.214
p.542/545 mutation vs wild-type	1.483 (0.347–6.342)	0.595	1.683 (0.220–12.867)	0.616
p.1047 mutation vs wild-type	0.873 (0.343–2.219)	0.776	2.502 (0.563–11.118)	0.228
Ki67 labeling index ≤30%				
PIK3CA mutation vs wild-type	1.672 (0.764–3.663)	0.199	2.977 (0.955–9.281)	0.600
Exon 9 mutation vs wild-type	0.556 (0.221–1.399)	0.213	1.040 (0.233–4.637)	0.959
Exon 20 mutation vs wild-type	2.838 (1.065–7.565)	**0.037**	3.099 (0.869–11.047)	0.081
p.542/545 mutation vs wild-type	0.464 (0.171–1.262)	0.133	1.480 (0.192–11.378)	0.706
p.1047 mutation vs wild-type	2.773 (0.958–8.027)	0.060	3.548 (0.805–15.642)	0.094

(*Continued*)

Table S4 (*Continued*)

Variables	PFS		OS	
	HR (95% CI)	*P*-value	HR (95% CI)	*P*-value
Ki67 labeling index >30%				
PIK3CA mutation vs wild-type	1.270 (0.546–2.951)	0.579	1.716 (0.689–4.274)	0.246
Exon 9 mutation vs wild-type	1.315 (0.398–4.343)	0.653	1.941 (0.458–8.234)	0.368
Exon 20 mutation vs wild-type	1.409 (0.492–4.039)	0.523	1.611 (0.552–4.705)	0.383
p.542/545 mutation vs wild-type	1.267 (0.384–4.186)	0.697	1.881 (0.443–7.977)	0.391
p.1047 mutation vs wild-type	1.478 (0.448–4.875)	0.521	1.661 (0.493–5.597)	0.413
Tumor stage I and II				
PIK3CA mutation vs wild-type	1.070 (0.557–2.059)	0.838	1.817 (0.808–4.086)	0.148
Exon 9 mutation vs wild-type	0.578 (0.247–1.349)	0.205	0.790 (0.270–2.314)	0.667
Exon 20 mutation vs wild-type	1.584 (0.744–3.370)	0.233	2.301 (0.904–5.853)	0.080
p.542/545 mutation vs wild-type	0.490 (0.194–1.237)	0.131	0.837 (0.248–2.825)	0.837
p.1047 mutation vs wild-type	1.572 (0.691–3.577)	0.281	2.510 (0.859–7.333)	0.092
Tumor stage III				
PIK3CA mutation vs wild-type	1.519 (0.498–4.635)	0.463	2.331 (0.514–10.581)	0.273
Exon 9 mutation vs wild-type	1.359 (0.309–5.979)	0.685	–	–
Exon 20 mutation vs wild-type	1.867 (0.428–8.137)	0.406	1.201 (0.265–5.442)	0.812
p.542/545 mutation vs wild-type	1.225 (0.277–5.414)	0.789	–	–
p.1047 mutation vs wild-type	1.498 (0.342–6.550)	0.592	0.911 (0.200–4.149)	0.904
Lymph node negative				
PIK3CA mutation vs wild-type	1.002 (0.423–2.372)	0.997	1.626 (0.606–4.360)	0.334
Exon 9 mutation vs wild-type	0.236 (0.085–0.655)	**0.006**	0.444 (0.127–1.545)	0.202
Exon 20 mutation vs wild-type	2.214 (0.742–6.612)	0.154	2.399 (0.766–7.513)	0.133
p.542/545 mutation vs wild-type	0.107 (0.029–0.403)	**0.001**	0.401 (0.090–1.790)	0.231
p.1047 mutation vs wild-type	3.065 (0.717–13.102)	0.131	3.120 (0.712–13.673)	0.131
Lymph node positive				
PIK3CA mutation vs wild-type	1.428 (0.681–2.996)	0.346	2.279 (0.848–6.124)	0.102
Exon 9 mutation vs wild-type	1.926 (0.586–6.328)	0.280	4.403 (0.593–32.711)	0.147
Exon 20 mutation vs wild-type	1.283 (0.558–2.951)	0.557	1.669 (0.570–4.892)	0.350
p.542/545 mutation vs wild-type	1.687 (0.514–5.540)	0.389	4.059 (0.546–30.159)	0.171
p.1047 mutation vs wild-type	1.132 (0.492–2.600)	0.771	1.471 (0.502–4.309)	0.482
Tumor size <5 cm				
PIK3CA mutation vs wild-type	1.074 (0.606–1.905)	0.806	1.584 (0.784–3.200)	0.200
Exon 9 mutation vs wild-type	0.619 (0.298–1.283)	0.197	1.101 (0.387–3.129)	0.857
Exon 20 mutation vs wild-type	1.530 (0.766–3.058)	0.228	1.602 (0.730–3.516)	0.240
p.542/545 mutation vs wild-type	0.553 (0.257–1.191)	0.130	1.216 (0.372–3.983)	0.746
p.1047 mutation vs wild-type	1.704 (0.801–3.623)	0.166	1.839 (0.767–4.410)	0.172
Tumor size ≥5 cm				
PIK3CA mutation vs wild-type	3.231 (0.407–25.614)	0.267	–	–
Exon 9 mutation vs wild-type	–	–	–	–
Exon 20 mutation vs wild-type	3.231 (0.407–25.614)	0.267	–	–
p.542/545 mutation vs wild-type	–	–	–	–
p.1047 mutation vs wild-type	0.286 (0.026–3.161)	0.307	–	–
Luminal and HER2 subtypes				
PIK3CA mutation vs wild-type	1.323 (0.752–2.327)	0.332	2.125 (1.032–4.375)	**0.041**
Exon 9 mutation vs wild-type	0.723 (0.351–1.488)	0.378	1.254 (0.443–3.550)	0.670
Exon 20 mutation vs wild-type	1.969 (0.988–3.923)	0.054	2.337 (1.024–5.331)	**0.044**
p.542/545 mutation vs wild-type	0.637 (0.298–1.361)	0.244	1.373 (0.421–4.478)	0.600
p.1047 mutation vs wild-type	1.945 (0.917–4.125)	0.083	2.493 (0.971–6.399)	0.058
Triple negative subtype				
PIK3CA mutation vs wild-type	0.720 (0.083–6.247)	0.766	0.720 (0.083–6.247)	0.766
Exon 9 mutation vs wild-type	–	–	–	–
Exon 20 mutation vs wild-type	0.255 (0.026–2.463)	0.237	0.255 (0.026–2.463)	0.237
p.542/545 mutation vs wild-type	–	–	–	–
p.1047 mutation vs wild-type	0.255 (0.026–2.463)	0.237	0.255(0.026–2.463)	0.237

Note: *P*<0.05 was considered statistically significant and those values are shown in bold.
Abbreviations: ER, estrogen receptor; HER2, human epidermal growth factor receptor 2; OS, overall survival; PFS, progression-free survival; PR, progesterone receptor.

Table S5 Comparison of association between *PIK3CA* mutations and various clinicopathological features in different areas in China

Authors	Year of publication	Area	No. of patients	*PIK3CA* mutation	Sample type	Methods	Association between PIK3CA mutations and clinicopathological characteristics
Hu et al[1]	2018	Changsha, Hunan	68	33.82%	Peripheral blood	Targeted NGS	*PIK3CA* mutations: worse progression-free survival
Deng et al[2]	2019	Chengdu, Sichun	507	46.5%	FTS	Targeted NGS	*PIK3CA* mutations: ER positive, PR positive, HER3 negative; two or three mutations in PIK3CA: poor prognosis for overall survival
Chen et al[3]	2018	Shanghai	149	43.6%	FTS	Targeted NGS	*PIK3CA* mutations: older age, ER positive, PR positive
Cheng et al[4]	2017	Luzhou, Sichun	32	28.12%	FFPE	DS	*PIK3CA* mutations: more invasiveness lymph node, bigger tumor size
Yuan et al[5]	2015	Beijing	729	28.3%	FTS	DS	*PIK3CA* mutations: ER positive, PR positive, less response to neoadjuvant chemotherapy
Wang et al[6]	2015	Xining, Qinghai	25	32%	FTS	DS	Not detected
Liu et al[7]	2015	Dalian, Liaoning	80	32.5%	FFPE	Targeted NGS	No significance
Deng et al[8]	2015	Chengdu, Sichun	288	15.6%	FFPE	DS	*PIK3CA* mutations: poor outcome of ER-positive breast cancer
Zhang et al[9]	2014	Beijing	93	32.3%	FTS	xTAG liquid chip	*PIK3CA* mutations: patients' clinical response to neoadjuvant chemotherapy
Bai et al[10]	2014	Xi'an Shaanxi	105	35.2%	FFPE	Targeted NGS	*PIK3CA* mutations: older age
Tong et al[11]	2012	Guangzhou, Guangdong	120	7.5%	FTS	MA	*PIK3CA* mutations: older age
Li et al[12]	2010	Shanghai	233	19.7%	FFPE	DS	*PIK3CA* mutations: high grade, ER positive, PR positive, PTEN positive
Current study	2018	Hefei, Anhui	494	38.06%	FFPE	DS	*PIK3CA* mutations: luminal and HER2 positive, ER positive, PR positive, low Ki67 index, better overall survival; exon 9 mutations: worse progression-free survival; exon 20 mutations: better progression-free survival and overall survival; H1047 mutations: older age, better progression-free survival and overall survival; non-hotspot mutations: larger tumor size

Abbreviations: DS, direct sequencing; ER, estrogen receptor; FFPE, formalin-fixed, paraffin-embedded tissue samples; FTS, frozen tissue samples; HER3, human epidermal growth factor receptor 3; MA, MassARRAY; NGS, next generation sequencing; PR, progesterone receptor.

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