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Association of *PIK3CA* Mutation Status before and after Neoadjuvant Chemotherapy with Response to Chemotherapy in Women with Breast Cancer

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Abstract

Purpose: The association between *PIK3CA* mutations and response to neoadjuvant chemotherapy in women with primary breast cancer is not fully elucidated.

Experimental Design: *PIK3CA* mutations in breast cancer tissues that were taken prior to the initiation of neoadjuvant chemotherapy were identified in 729 operable primary breast cancer patients who received neoadjuvant chemotherapy. Among these, the *PIK3CA* mutations were also reassessed in tumor tissues procured following operation in 102 patients after completion of neoadjuvant chemotherapy.

Results: A total of 206 out of 729 (28.3%) patients had *PIK3CA* mutations, and 19.5% of patients (142/729) in this cohort achieved a pathologic complete response (pCR) after neoadjuvant chemotherapy. Patients with *PIK3CA* mutations exhibited a lower pCR rate than did those with wild-type (14.6% vs. 21.4%, $P = 0.035$). No significant differences in disease-free survival (DFS) or distant disease-free survival (DDFS) were observed between *PIK3CA* mutant and wild-type in the entire study population. Among the 102 patients with *PIK3CA* mutation statuses available before and after neoadjuvant chemotherapy, 24 patients (23.5%) had *PIK3CA* mutations before neoadjuvant chemotherapy. Of these 24 patients, 15 patients retained their initial *PIK3CA* mutations and 9 patients lost their initial mutations after neoadjuvant chemotherapy. Patients who retained the initial mutations after neoadjuvant chemotherapy ($n = 15$) had a worse DDFS than the remaining patients ($n = 87$) in this subgroup [unadjusted HR, 2.34; 95% confidence interval (CI), 0.98–5.62; $P = 0.050$].

Conclusions: Patients with *PIK3CA* mutations are less likely to respond to neoadjuvant chemotherapy. Patients who retain their initial *PIK3CA* mutations after neoadjuvant chemotherapy have an unfavorable survival. *Clin Cancer Res; 21(19); 4365–72. ©2015 AACR.*

Introduction

Approximately 30% of breast cancer patients carry *PIK3CA* (phosphatidylinositol-4, 5-bisphosphate 3-kinase, catalytic subunit alpha) mutations (1–6). More than 90% of the *PIK3CA* mutations in breast cancers occur in three hotspots of the *PIK3CA* gene: E542 and E545 in the helical domain (exon 9) and H1047 in the kinase domain (exon 20; refs. 2, 7–11). All three hotspot mutations are oncogenic and constitutively activate the PI3K pathway (3, 12–20). The remaining mutations are distributed over the entire *PIK3CA*-coding sequence.

The role of *PIK3CA* mutations in breast cancer survival is controversial (21–31). Many studies suggested that *PIK3CA* mutations are associated with favorable survival (21–26), others found that *PIK3CA* mutations are associated with worse outcomes (27, 28), and a number of studies found no association between *PIK3CA* mutations and breast cancer survival (29–31).

Recent *in vitro* studies suggested that tumor cells with an activated PI3K pathway are less sensitive to chemotherapy agents (32, 33). However, few studies have investigated the association between *PIK3CA* mutations and the response to neoadjuvant chemotherapy in breast cancer (19, 34, 35). Therefore, whether breast cancer patients with *PIK3CA* mutations are sensitive to neoadjuvant chemotherapy is not fully determined.

In the current study, the primary objective was to investigate the association between *PIK3CA* mutations and the response to neoadjuvant chemotherapy in operable primary breast cancer patients who received neoadjuvant chemotherapy ($n = 729$), and to explore the association between the *PIK3CA* mutations and survival in this cohort. The second objective was to identify the *PIK3CA* mutations in breast cancer tissues before and after neoadjuvant chemotherapy in a subgroup of patients ($n = 102$) in this cohort and to investigate whether changes in *PIK3CA* mutations during treatment influenced the clinical outcome of this subgroup.

Patients and Methods

Study population

Fresh breast cancer tissues obtained from a 14-gauge core-needle biopsy prior to neoadjuvant chemotherapy were available for 774 operable primary breast cancer patients (stages I–III) who received neoadjuvant chemotherapy at the Breast Center, Peking

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Note: Supplementary data for this article are available at Clinical Cancer Research Online (http://clincancerres.aacrjournals.org/).

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Translational Relevance

The association of *PIK3CA* mutations before and after neoadjuvant chemotherapy with response to chemotherapy in women with primary breast cancer is not fully elucidated. In the current study, we found that patients with *PIK3CA* mutations exhibited a lower pathologic complete response rate than did those with wild-type. Among the 102 patients that the *PIK3CA* mutation status was available before and after neoadjuvant chemotherapy, 24 of 102 patients (23.5%) carried *PIK3CA* mutations before neoadjuvant chemotherapy. Nine patients lost their initial mutations after neoadjuvant chemotherapy. Patients who retained the initial mutations after neoadjuvant chemotherapy had a worse survival than those with wild-type or those who lost their initial *PIK3CA* mutations. Our findings suggest that patients with *PIK3CA* mutations are less likely to respond to neoadjuvant chemotherapy; furthermore, patients who retain their initial mutations after neoadjuvant chemotherapy harbor an aggressive phenotype, and those patients may be suitable for *PIK3CA*-targeted therapy.

University Cancer Hospital (Beijing, China) from May 2004 to May 2011. *PIK3CA* mutations were successfully identified in fresh cancer tissues that were taken before neoadjuvant chemotherapy in 729 of 774 patients (94.2%). Of these 729 patients, breast cancer tissues were also available for 102 patients after neoadjuvant chemotherapy, and *PIK3CA* mutations were successfully identified in these 102 patients.

The patients' ages at diagnosis ranged from 25 to 73 years, with a median of 49 years. The tumors were graded according to the modified Bloom–Richardson system. Tumor stage was classified according to the tumor–node–metastasis (TNM) classification of the Union Internationale Contre le Cancer. Tumor size was defined as the maximum tumor diameter measured by ultrasound at the time of diagnosis.

Follow-up data were available for all 729 patients, the median length of follow-up was 66 months (range, 5–113 months). During the follow-up period, 143 patients experienced a local or distant recurrence or died of the disease. The study was approved by the Research and Ethical Committee of Peking University Cancer Hospital.

PIK3CA mutation analysis

Total RNA was extracted from breast tumor tissues obtained from a core-needle biopsy prior to neoadjuvant chemotherapy or taken following surgery using TRizol reagent (Life Technologies Inc.) according to the manufacturer's instructions. In brief, 500 ng of RNA was transcribed to cDNA by a reverse transcriptase in a total of 20 μL reverse transcription reaction solution containing 4.0 μL 5× first strand buffer, 10 mmol/L DTT, 20 U RNase inhibitor, 1 mmol/L dNTP, 400 ng random primer and 200 U superscript II reverse transcriptase (Invitrogen). The resulting cDNA was subjected to PCR amplification. Mutational analysis of *PIK3CA* was performed using a set of 8 primer pairs (Supplementary Table S1) that covered the entire coding region of the *PIK3CA* gene. All fragments were sequenced using the BigDye Terminator Cycle Sequencing Kit and ABI3730 automated sequencer (Applied Biosystems). Each mutation was confirmed in duplicate.

Pathology

Estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor-2 (HER2) statuses were determined using the breast cancer tissues obtained from the core-needle biopsy using an immunohistochemical (IHC) assay, as described previously (36). ER or PR was considered positive when $\geq$1% of tumor cells showed positive nuclear staining. HER2 positivity was defined as a score of 3+ (IHC) or by HER2 gene amplification (fluorescence *in situ* hybridization). In this study, the breast cancer subgroups were classified according to ER, PR, and HER2 statuses: luminal (ER^+ and/or PR^+, $HER2^-$), $HER2^+$, and triple-negative (TN, $ER^-/PR^-/HER2^-$; ref. 30).

Treatment

All the 729 patients received neoadjuvant chemotherapy, and 93% of the patients received 4 to 8 cycles. Treatments were categorized into four subgroups as follows:

1. A total of 265 patients received anthracycline based regimens. Of these, 184 patients received a 5-fluorouracil, pirarubicin, and cyclophosphamide (CTF) regimen, the details of which are described previously (36); 56 patients received the FEC regimen, epirubicin 90 to 100 mg/m^2 on day 1, every 3 weeks, the doses of 5-fluorouracil and cyclophosphamide were identical to the CTF regimen; 23 patients received the CAF regimen; 1 patient received pirarubicin plus cyclophosphamide regimen; and 1 patient received the doxorubicin and cyclophosphamide (AC) regimen.
2. A total of 317 patients received anthracycline–taxane-based regimens. Of these, 131 patients received 2 cycles of anthracycline regimens followed by 4 cycles of paclitaxel alone (80 mg/m^2 i.v. once a week for 12 weeks) or docetaxel plus cyclophosphamide (docetaxel 75 mg/m^2 i.v. on day 1, cyclophosphamide 600 mg/m^2 i.v. on day 1, every 3 weeks). One hundred and eighty-six patients received 2 cycles of anthracycline regimens followed by paclitaxel plus carboplatin (paclitaxel 175 mg/m^2 i.v. on day 1, or paclitaxel 60 mg/m^2 i.v. on days 1, 8, and 15, carboplatin AUC6, i.v. on day 1, every 3 weeks).
3. Taxane-based regimens: 100 patients received 4 cycles of paclitaxel alone (paclitaxel 60 mg/m^2 i.v. on days 1, 8, and 15, every 3 weeks).
4. Other regimens: the remaining 47 patients in this cohort of 729 patients received other regimens.

A total of 222 patients had $HER2^+$ tumors. Of these, 41 women were treated with neoadjuvant trastuzumab in combination with one of the above-described regimens. pCR was defined as no invasive breast cancer cells in the breast after completion of neoadjuvant chemotherapy (37).

Approximately 59.2% of the patients received adjuvant chemotherapy after surgery. Of these, 55.3% of patients with *PIK3CA* mutations and 60.6% of patients with wild-type received adjuvant chemotherapy after operation, respectively. In addition, patients with axillary positive lymph nodes ($\geq$3 nodes) or who had breast-conserving surgery received radiotherapy, and patients with ER- and/or PR-positive disease received endocrine therapy.

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Statistical analysis

The associations between *PIK3CA* mutations and clinicopathologic characteristics or pCR rate were performed using the Pearson χ^2 test or the Fisher exact test. For the survival analyses, disease-free survival (DFS) was defined as the time from the date of diagnosis to first recurrence (local or distant), the contralateral breast cancer, or death from breast cancer without a recorded relapse. Distant disease-free survival (DDFS) was defined as the time from the date of diagnosis to either the first distant recurrence or death for which breast cancer was the primary or underlying cause. Survival was estimated using the Kaplan–Meier product-limit method, and differences were tested for statistical significance using the log-rank test. Cox proportional hazards regression models were used to test the prognostic role of the *PIK3CA* mutation status [HR and 95% confidence intervals (CI)]. Two-sided *P* values less than 0.05 were considered to be statistically significant. All analyses were performed using the SPSS Statistics 20.0 software.

Results

The frequency of PIK3CA mutations

Of these patients, 28.3% (206/729) had *PIK3CA* mutations. The frequencies of *PIK3CA* mutations in the luminal, HER2$^+$, and TN subgroups were 30.6%, 27.5%, 22.7%, respectively ($P = 0.22$; Supplementary Table S2). *PIK3CA* mutant than wild-type were more likely to be ER- and PR-positive (ER: $P = 0.034$; PR: $P = 0.007$, respectively), and no significant association was found between *PIK3CA* mutations and other clinicopathologic characteristics (Table 1).

Six patients contained two *PIK3CA* mutations; therefore, 212 *PIK3CA* mutations were found in the 206 patients. Of these 212 mutations, 87.3% (185/212) clustered in the three hotspots (E542/E545 and H1047), and 12.7% (27/212) clustered in non-hotspots.

Response to neoadjuvant chemotherapy

In total, 19.5% of patients (142/729) achieved a pCR after neoadjuvant chemotherapy. Patients with *PIK3CA* mutations had

Table 1. Association of patient/tumor characteristics with *PIK3CA* mutation status

Characteristic	Total (*n*)	Wild-type (*n* = 523) *n* (%)	Mutant (*n* = 206) *n* (%)	*P*[a]
Age				
≤50 years	395	293 (56.0)	102 (49.5)	0.11
>50 years	334	230 (44.0)	104 (50.5)	
Tumor size				
≤2 cm	233	173 (33.1)	60 (29.1)	0.30
>2 cm	496	350 (66.9)	146 (70.9)	
Tumor grade				
I	49	34 (6.7)	15 (7.4)	0.11
II	545	382 (74.9)	163 (80.7)	
III	118	94 (18.4)	24 (11.9)	
Unknown	17	13	4	
Lymph nodes status				
Negative	377	263 (50.7)	114 (55.9)	0.21
Positive	346	256 (49.3)	90 (44.1)	
Unknown	6	4	2	
ER status				
Negative	245	188 (36.1)	57 (27.8)	0.034
Positive	481	333 (63.9)	148 (72.2)	
Unknown	3	2	1	
PR status				
Negative	323	248 (48.2)	75 (36.9)	0.007
Positive	395	267 (51.8)	128 (63.1)	
Unknown	11	8	3	
HER2 status				
Negative	505	361 (69.2)	144 (70.2)	0.76
Positive	222	161 (30.8)	61 (29.8)	
Unknown	2	1	1	
Subgroup				
Luminal	376	261 (50.1)	115 (56.1)	0.22
HER2$^+$	222	161 (30.9)	61 (29.8)	
TN	128	99 (19.0)	29 (14.1)	
Unknown	3	2	1	
Regimens				
Anthracycline with or without taxane	582	424 (81.1)	158 (76.7)	0.42
Taxane	135	91 (17.4)	44 (21.4)	
Others	12	8 (1.5)	4 (1.9)	
Trastuzumab use				
No	688	498 (95.2)	190 (92.2)	0.12
Yes	41	25 (4.8)	16 (7.8)	
Surgery type				
BCS	299	214 (40.9)	85 (41.3)	0.93
Mastectomy	430	309 (59.1)	121 (58.7)	

NOTE. Luminal subgroup: ER$^+$ and/or PR$^+$, HER2$^-$; TN subgroup: ER$^-$/PR$^-$/HER2$^-$.
Abbreviation: BCS, breast-conserving surgery.
[a]*PIK3CA* mutant compared with wild-type.

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Table 2. Pathologic complete response rates by *PIK3CA* status in the entire cohort of 729 patients

PIK3CA mutation status	Total (*n*)	Non-pCR, *n* (%)	pCR, *n* (%)	*P*[a]
Mutant	206	176 (85.4)	30 (14.6)	0.035
Wild-type	523	411 (78.6)	112 (21.4)	
Total	729	587 (80.5)	142 (19.5)	

[a]*PIK3CA* mutant compared with wild-type.

a lower pCR rate than those with wild-type (pCR rate, 14.6% vs 21.4%, $P = 0.035$; Table 2). In multivariate analysis, *PIK3CA* mutation status tended to be an unfavorable factor for pCR (OR, 0.68; 95% CI, 0.42–1.11; $P = 0.13$; Supplementary Table S3). Patients with *PIK3CA* mutations located in the three hotspots (E542/E545 and H1047) had a lower pCR rate than patients with wild-type (13.5% vs 21.4%, $P = 0.019$; Supplementary Table S4). No significant difference in pCR rate between patients with non-hotspot mutations and patients with wild-type was observed (23.8% vs. 21.4%, $P = 0.79$; Supplementary Table S4). The pCR rate for patients with E542/E545 mutations was similar to those with H1047 mutations (12.5% vs 14.0%, $P = 0.68$; Supplementary Table S5). However, patients with H1047 mutation showed a trend for better survival than those with E542/E545 mutation (DFS: unadjusted HR, 0.71; 95% CI, 0.39–1.29, $P = 0.25$; DDFS: unadjusted HR, 0.60; 95% CI, 0.31–1.16, $P = 0.13$; Supplementary Fig. S1). When the pCR includes both breast and axillary lymph nodes, *PIK3CA* mutations were associated with lower pCR rate in the entire study cohort (mutant vs. wild-type, 13.6% vs. 18.2%, $P = 0.14$), although the difference did not reach a significance.

Of the patients treated with anthracycline-based regimens ($n = 265$), patients with *PIK3CA* mutations had a lower pCR rate than those with wild-type (14.1% vs. 19.4%, $P = 0.33$; Table 3); among patients treated with anthracyclin–taxane-based regimens ($n = 317$), patients with *PIK3CA* mutations had a significantly lower pCR rate than those with wild-type (11.7% vs. 24.7%, $P = 0.009$; Table 3); among patients treated with taxane-based regimen ($n = 100$), the pCR rate was similar in patients with or without *PIK3CA* mutations (16.1% vs. 18.8%, $P = 0.74$; Table 3).

In the luminal subgroup ($n = 376$), patients with *PIK3CA* mutations had a lower pCR rate than those with wild-type (6.1% vs. 13.0%, $P = 0.047$; Supplementary Table S6). In the TN subgroup ($n = 128$), patients with *PIK3CA* mutations had a lower pCR rate than those without mutations (27.6% vs 37.4%, $P = 0.33$), although the difference did not reach a significance (Supplementary Table S6). The pCR rate was identical between *PIK3CA* mutant and wild-type among the $HER2^+$ patients who did not receive trastuzumab treatment ($n = 181$; 20.0% vs. 19.9%, $P = 0.98$); among the $HER2^+$ patients who received neoadjuvant trastuzumab ($n = 41$), patients with *PIK3CA* mutations had a lower pCR rate than those with wild-type (37.5% vs. 56.0%), but this difference was not significant ($P = 0.25$; Supplementary Table S6).

PIK3CA mutation status before and after neoadjuvant chemotherapy

The *PIK3CA* mutation status before and after neoadjuvant chemotherapy in 102 patients in this cohort was assessed. Of these, 24 patients (23.5%) had *PIK3CA* mutations before neoadjuvant chemotherapy (detailed information on these patients is presented in Table 4). After neoadjuvant chemotherapy, of these 24 patients, 15 patients retained their initial *PIK3CA* mutations (referred to as the mt-mt group), and these 15 patients mainly exhibited luminal subtype (Supplementary Table S7); 9 patients lost their initial mutations (referred to as the mt-wt group; Table 4). The *PIK3CA* status remained stable in the 78 patients with wild-type after neoadjuvant chemotherapy (referred to as the wt-wt group). Patients who retained their initial mutations had a lower pCR rate than those who lost their initial mutations (0.0% vs. 33.0%, $P = 0.042$; Table 5). Patients who retained their initial mutations after neoadjuvant chemotherapy had a borderline worse survival than those who lost their mutations or those with wild-type (DFS: unadjusted HR, 2.32; 95% CI, 0.97–5.57; $P = 0.052$; and DDFS: unadjusted HR, 2.34; 95% CI, 0.98–5.62; $P = 0.050$; Fig. 1). In multivariate analysis, retained the initial mutation remained as a nonsignificantly unfavorable factor (DFS: adjusted HR, 2.27; 95% CI, 0.83–6.25; $P = 0.11$; and DDFS: adjusted HR, 1.32; 95% CI, 0.92–1.90; $P = 0.14$; Supplementary Table S8).

Survival

The 5-year DFS and 5-year DDFS rates in the entire study population ($n = 729$) were 81.3% and 84.4%, respectively. The 5-year DFS in *PIK3CA* mutant and wild-type were 79.4% and 82.0%, respectively, and the 5-year DDFS in *PIK3CA* mutant and wild-type were 82.8% and 85.0%, respectively. In the univariate analysis, there were no significant differences in DFS and DDFS between the *PIK3CA* mutant and wild-type in the entire study population (DFS: unadjusted HR, 1.25; 95% CI, 0.88–1.77; $P = 0.21$; and DDFS: unadjusted HR, 1.18; 95% CI, 0.81–1.74; $P = 0.39$; Supplementary Fig. S2).

The association between the *PIK3CA* mutations and survival in different subgroups was also analyzed. Patients with *PIK3CA* mutations had a slightly worse survival than those with wild-type in the luminal subgroup (DFS: unadjusted HR, 1.30; 95% CI, 0.76–2.23; $P = 0.33$; and DDFS: unadjusted HR, 1.55; 95% CI, 0.89–2.69; $P = 0.12$; Supplementary Fig. S3).

Among the $HER2^+$ patients ($HER2^+$ subgroup) who did not receive trastuzumab treatment, *PIK3CA* mutant had a slightly better survival than wild-type (DFS: unadjusted HR, 0.82; 95% CI, 0.42–1.60; $P = 0.55$; and DDFS: unadjusted HR, 0.51; 95% CI, 0.21–1.22; $P = 0.13$; Supplementary Fig. S4). Among the $HER2^+$ patients who received trastuzumab treatment, patients with *PIK3CA* mutations had a tendency toward worse survival than those with wild-type (DFS: unadjusted HR, 3.34; 95% CI, 0.61–18.27; $P = 0.14$; DDFS: unadjusted HR, 4.73; 95% CI, 0.49–45.46; $P = 0.14$; Supplementary Fig. S5).

Table 3. Pathologic complete response rates by *PIK3CA* status in different neoadjuvant regimens

Regimens	Total (*n*)	Non-pCR, *n* (%)	pCR, *n* (%)	*P*[a]
Anthracycline ($n = 265$)				
Mutant	64	55 (85.9)	9 (14.1)	0.33
Wild-type	201	162 (80.6)	39 (19.4)	
Anthracycline-taxane ($n = 317$)				
Mutant	94	83 (88.3)	11 (11.7)	0.009
Wild-type	223	168 (75.3)	55 (24.7)	
Taxane ($n = 100$)				
Mutant	31	26 (83.9)	5 (16.1)	0.74
Wild-type	69	56 (81.2)	13 (18.8)	
Others ($n = 47$)				
Mutant	17	12 (70.6)	5 (29.4)	0.46
Wild-type	30	25 (83.3)	5 (16.7)	

[a]*PIK3CA* mutant compared with wild-type.

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Table 4. Twenty-four patients who had *PIK3CA* mutations before neoadjuvant chemotherapy and changes in the *PIK3CA* mutations after neoadjuvant chemotherapy

ID	*PIK3CA* status before NCT	*PIK3CA* status after NCT	Tumor cell percentage after NCT (%)	Tumor type	ER status	PR status	HER2 status	Regimens	Pathologic response
139	H1047R	WT	50	IDC	+	+	−	TP	Non-pCR
185	H1047R	WT	70	IDC	−	−	+	A	Non-pCR
544	H1047R	WT	10	IDC	+	+	−	A-TP	Non-pCR
557	Q815R	WT	50	IDC	+	−	−	A-TP	Non-pCR
717	E545K	WT	0	ILC	+	−	−	A-TP	pCR
779	E1064fs	WT	0	IDC	+	+	−	TP	pCR
990	H1047R	WT	30	IDC	−	−	−	A-T	Non-pCR
1057	H1047R	WT	20	IDC	+	+	−	A	Non-pCR
1416	E545K	WT	0	IDC	+	−	−	T	pCR
710	N345K	N345K	70	IDC	+	+	−	A-TP	Non-pCR
915	E545K	E545K	60	IDC	+	+	−	T	Non-pCR
1015	E545K	E545K	40	IDC	+	+	−	A-T	Non-pCR
1132	E545K	E545K	70	IDC	+	−	+	A	Non-pCR
360	E542K	E542K	20	IDC	+	+	−	A	Non-pCR
1556	E542K	E542K	40	IDC	+	+	−	T	Non-pCR
306	H1047R, Q815R	H1047R, Q815R	60	IDC	+	+	−	A-TP	Non-pCR
505	H1047R	H1047R	30	IDC	+	+	+	A-TP	Non-pCR
653	H1047R	H1047R	50	IDC	+	+	−	A-TP	Non-pCR
663	H1047R	H1047R	70	IDC	+	+	−	A-TP	Non-pCR
989	H1047R	H1047R	50	IDC	+	+	−	A-T	Non-pCR
994	H1047R	H1047R	50	IDC	−	−	+	A	Non-pCR
467	H1047L	H1047L	80	IDC	−	−	−	A-T	Non-pCR
878	H1047L	H1047L	40	IDC	+	+	−	A-T	Non-pCR
1541	H1047L	H1047L	30	IDC	+	+	−	A	Non-pCR

Abbreviations: NCT, neoadjuvant chemotherapy; IDC, invasive ductal carcinoma; ILC, invasive lobular carcinoma; A, anthracycline; T, taxane; TP, taxane plus carboplatin.

In the TN subgroup, *PIK3CA* mutant had a significantly worse DFS and non-significantly worse DDFS than wild-type (DFS: unadjusted HR, 2.11; 95% CI, 1.00–4.43; $P = 0.044$; and DDFS: unadjusted HR, 1.69; 95% CI, 0.73–3.91; $P = 0.22$; Supplementary Fig. S6).

Discussion

To our knowledge, the current study is one of the largest studies to investigate the association between *PIK3CA* mutations and response to neoadjuvant chemotherapy in breast cancer to date. We found that patients with *PIK3CA* mutations had a significantly lower pCR rate than those with wild-type. These findings suggested that *PIK3CA* mutant are resistant to neoadjuvant chemotherapy.

We explored the *PIK3CA* mutation status in 102 patients before and after neoadjuvant chemotherapy. Patients who retained their initial *PIK3CA* mutations showed a worse survival than those who lost their initial mutations or those with wild-type. These findings suggested that patients who retained their initial mutations after neoadjuvant chemotherapy harbored an aggressive phenotype and were less sensitive to chemotherapy; therefore, those patients might be suitable for *PIK3CA*-targeted therapy, as one recent study indicated that breast or gynecologic cancer patients with *PIK3CA* mutations have a higher response to PI3K/AKT/mTOR inhibitors than those with wild-type (11). On the other hand, patients who lost their initial mutations after neoadjuvant chemotherapy had a favorable outcome and benefited from neoadjuvant chemotherapy.

Table 5. Pathologic complete response rates in 102 patients whose *PIK3CA* mutation status was available before and after neoadjuvant chemotherapy

Subgroup	Total (*n*)	Non-pCR, *n* (%)	pCR, *n* (%)	*P*[a]
mt-mt	15	15 (100.0)	0 (0.0)	0.042
wt-wt	78	66 (84.6)	12 (15.4)	
mt-wt	9	6 (66.7)	3 (33.3)	
Total	102	87 (85.3)	15 (14.7)	

[a]mt-mt compared with mt-wt.

The three hotspot mutations (E542/E545 and H1047) accounted for the majority of mutations of the *PIK3CA* gene in the current study. The mechanisms that activate the PI3K/AKT pathway may be different between the E542/E545 mutations and the H1047 mutation (13–17,23, 24). Janku and colleagues recently reported that patients with H1047 mutation are more sensitive to a PI3K/AKT/mTOR inhibitor than those with other mutations or patients with wild-type, and patients with H1047 mutation show a trend for a longer progression-free survival than those with other mutations (38, 39). In our present study, although the E542/E545 and H1047 mutations exhibited a similar efficacy in response to neoadjuvant chemotherapy, patients with H1047 mutation had a nonsignificantly better survival than those with E542/E545 mutation.

Emerging evidence suggested that *PIK3CA* mutations have different roles in different subgroups when stratified by ER, PR, and HER2 status (25, 29, 40, 41). We found that the frequency of *PIK3CA* mutations was higher for luminal subgroup than for the $HER2^+$ and TN subgroups, and this observation is consistent with those of previous studies (24, 26). *PIK3CA* mutations were significantly associated with a lower pCR rate in the luminal subgroup, whereas *PIK3CA* mutations were associated with a lower pCR rate in the TN subgroup, although the difference was not significant. *PIK3CA* mutations were not associated with pathologic response among the $HER2^+$ patients treated with neoadjuvant chemotherapy without trastuzumab. Our results suggested that the association between mutated

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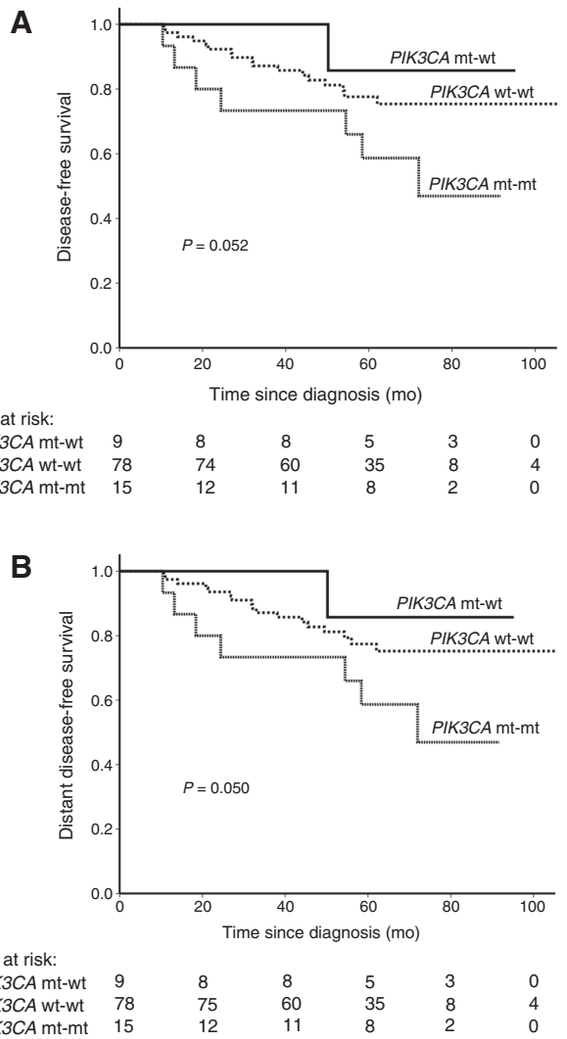

Figure 1.
Kaplan–Meier estimates of DFS and DDFS according to the *PIK3CA* mutation status in 102 patients whose *PIK3CA* mutation status was available before and after neoadjuvant chemotherapy. A, DFS; B, DDFS.

PIK3CA and a reduced pCR rate is dependent on the molecular types, and the association is more pronounced in the luminal subgroup.

In contrast, the HER2$^+$ patients treated with neoadjuvant chemotherapy in combination with trastuzumab, we found that patients with *PIK3CA* mutations had a lower pCR rate than those with wild-type. Interestingly, two recent studies have demonstrated that HER2$^+$ breast cancer patients with *PIK3CA* mutations are less likely to achieve a pCR compared with those with wild-type when the patients treated with neoadjuvant chemotherapy plus anti-HER2 therapies in the GeparQuattro, GeparQuinto, and GeparSixto trials (19) and the NeoATTO trial (35). Similarly, *PIK3CA* mutation is associated with a shorter progression-free survival in HER2$^+$ metastatic breast cancer treated with HER2-targeted therapies in the CLEOPATRA trial (42). Our present observation is in agreement with these findings. However, unlike the results observed in the neoadjuvant and metastatic settings, there is no association between the *PIK3CA* mutation and the degree of benefit from transtuzumab in adjuvant setting in the NSABP B-31 trial (43).

Although patients with *PIK3CA* mutations had a slightly poorer DFS and DDFS than those with wild-type in the entire study population ($n = 729$), the differences did not reach a significance. However, *PIK3CA* mutations were associated with worse DFS in the TN subgroup. Due to the relatively small sample size of the TN subgroup ($n = 128$), further independent studies are warranted to confirm this observation.

There are two limitations to our study. Although the overall cohort was large, the number of patients who had matched tumor tissues before and after neoadjuvant chemotherapy was not large ($n = 102$); in particular, the *PIK3CA* mutant in this group were relatively small. The current study was retrospective, and the neoadjuvant chemotherapy regimens were not assigned at randomized. Therefore, caution is required when interpreting our results.

In summary, patients with *PIK3CA* mutations are less likely to respond to neoadjuvant chemotherapy. Although *PIK3CA* mutations are not associated with survival in the entire study population, patients who retain their initial *PIK3CA* mutations after neoadjuvant chemotherapy have a lower pCR rate and an unfavorable survival when compared with those who lose their initial *PIK3CA* mutations.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Authors' Contributions

Conception and design: Y. Xie
Development of methodology: H. Yuan, J. Chen, Y. Liu
Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): T. Ouyang, T. Wang, Z. Fan, T. Fan, B. Lin, Y. Xie
Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): H. Yuan, Y. Liu, Y. Xie
Writing, review, and/or revision of the manuscript: H. Yuan, Y. Xie
Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): T. Ouyang, J. Li, T. Wang, Y. Xie
Study supervision: J. Li, Y. Xie

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References

1. The Cancer Genome Atlas Network. Comprehensive molecular portraits of human breast tumours. Nature 2012;490:61–70.
2. Vogelstein B, Papadopoulos N, Velculescu VE, Zhou S, Diaz LA Jr, Kinzler KW. Cancer genome landscapes. Science 2013;339:1546–58.

3. Guarneri V, Generali DG, Frassoldati A, Artioli F, Boni C, Cavanna L, et al. Double-blind, placebo-controlled, multicenter, randomized, phase IIb neoadjuvant study of letrozole-lapatinib in postmenopausal hormone receptor-positive, human epidermal growth factor receptor 2-negative, operable breast cancer. J Clin Oncol 2014;32:1050–7.
4. Miller TW, Balko JM, Arteaga CL. Phosphatidylinositol 3-kinase and antiestrogen resistance in breast cancer. J Clin Oncol 2011;29: 4452–61.
5. Liu P, Cheng H, Roberts TM, Zhao JJ. Targeting the phosphoinositide 3-kinase pathway in cancer. Nat Rev Drug Discov 2009;8:627–44.
6. Campbell IG, Russell SE, Choong DY, Montgomery KG, Ciavarella ML, Hooi CS, et al. Mutation of the PIK3CA gene in ovarian and breast cancer. Cancer Res 2004;64:7678–81.
7. Samuels Y, Wang Z, Bardelli A, Silliman N, Ptak J, Szabo S, et al. High frequency of mutations of the PIK3CA gene in human cancers. Science 2004;304:554.
8. Janku F, Hong DS, Fu S, Piha-Paul SA, Naing A, Falchook GS, et al. Assessing PIK3CA and PTEN in early-phase trials with PI3K/AKT/mTOR inhibitors. Cell Rep 2014;6:377–87.
9. Beelen K, Opdam M, Severson TM, Koornstra RH, Vincent AD, Wesseling J, et al. PIK3CA mutations, phosphatase and tensin homolog, human epidermal growth factor receptor 2 and insulin-like growth factor 1 receptor and adjuvant tamoxifen resistance in postmenopausal breast cancer patients. Breast Cancer Res 2014;16:R13.
10. Engelman JA. Targeting PI3K signalling in cancer: opportunities, challenges and limitations. Nat Rev Cancer 2009;9:550–62.
11. Janku F, Wheler JJ, Westin SN, Moulder SL, Naing A, Tsimberidou AM, et al. PI3K/AKT/mTOR inhibitors in patients with breast and gynecologic malignancies harboring PIK3CA mutations. J Clin Oncol 2012;30: 777–82.
12. Liu P, Cheng H, Santiago S, Raeder M, Zhang F, Isabella A, et al. Oncogenic PIK3CA-driven mammary tumors frequently recur via PI3K pathway-dependent and PI3K pathway-independent mechanisms. Nat Med 2011; 17:1116–20.
13. Zhao L, Vogt PK. Helical domain and kinase domain mutations in p110alpha of phosphatidylinositol 3-kinase induce gain of function by different mechanisms. Proc Natl Acad Sci U S A 2008;105: 2652–7.
14. Miled N, Yan Y, Hon WC, Perisic O, Zvelebil M, Inbar Y, et al. Mechanism of two classes of cancer mutations in the phosphoinositide 3-kinase catalytic subunit. Science 2007;317:239–42.
15. Yuan W, Stawiski E, Janakiraman V, Chan E, Durinck S, Edgar KA, et al. Conditional activation of Pik3ca(H1047R) in a knock-in mouse model promotes mammary tumorigenesis and emergence of mutations. Oncogene 2013;32:318–26.
16. Huang CH, Mandelker D, Schmidt-Kittler O, Samuels Y, Velculescu VE, Kinzler KW, et al. The structure of a human p110alpha/p85alpha complex elucidates the effects of oncogenic PI3Kalpha mutations. Science 2007; 318:1744–8.
17. Hao Y, Wang C, Cao B, Hirsch BM, Song J, Markowitz SD, et al. Gain of interaction with IRS1 by p110alpha-helical domain mutants is crucial for their oncogenic functions. Cancer Cell 2013;23:583–93.
18. Mayer IA, Abramson VG, Isakoff SJ, Forero A, Balko JM, Kuba MG, et al. Stand up to cancer phase Ib study of pan-phosphoinositide-3-kinase inhibitor buparlisib with letrozole in estrogen receptor-positive/human epidermal growth factor receptor 2-negative metastatic breast cancer. J Clin Oncol 2014;32:1202–9.
19. Loibl S, von Minckwitz G, Schneeweiss A, Paepke S, Lehmann A, Rezai M, et al. PIK3CA mutations are associated with lower rates of pathologic complete response to anti-human epidermal growth factor receptor 2 (her2) therapy in primary HER2-overexpressing breast cancer. J Clin Oncol 2014;32:3212–20.
20. Dave B, Migliaccio I, Gutierrez MC, Wu MF, Chamness GC, Wong H, et al. Loss of phosphatase and tensin homolog or phosphoinositol-3 kinase activation and response to trastuzumab or lapatinib in human epidermal growth factor receptor 2-overexpressing locally advanced breast cancers. J Clin Oncol 2011;29:166–73.
21. Perez-Tenorio G, Alkhori L, Olsson B, Waltersson MA, Nordenskjold B, Rutqvist LE, et al. PIK3CA mutations and PTEN loss correlate with similar prognostic factors and are not mutually exclusive in breast cancer. Clin Cancer Res 2007;13:3577–84.
22. Maruyama N, Miyoshi Y, Taguchi T, Tamaki Y, Monden M, Noguchi S. Clinicopathologic analysis of breast cancers with PIK3CA mutations in Japanese women. Clin Cancer Res 2007;13:408–14.
23. Barbareschi M, Buttitta F, Felicioni L, Cotrupi S, Barassi F, Del Grammastro M, et al. Different prognostic roles of mutations in the helical and kinase domains of the PIK3CA gene in breast carcinomas. Clin Cancer Res 2007;13:6064–9.
24. Kalinsky K, Jacks LM, Heguy A, Patil S, Drobnjak M, Bhanot UK, et al. PIK3CA mutation associates with improved outcome in breast cancer. Clin Cancer Res 2009;15:5049–59.
25. Cizkova M, Susini A, Vacher S, Cizeron-Clairac G, Andrieu C, Driouch K, et al. PIK3CA mutation impact on survival in breast cancer patients and in ERalpha, PR and ERBB2-based subgroups. Breast Cancer Res 2012;14: R28.
26. Loi S, Haibe-Kains B, Majjaj S, Lallemand F, Durbecq V, Larsimont D, et al. PIK3CA mutations associated with gene signature of low mTORC1 signaling and better outcomes in estrogen receptor-positive breast cancer. Proc Natl Acad Sci U S A 2010;107:10208–13.
27. Li SY, Rong M, Grieu F, Iacopetta B. PIK3CA mutations in breast cancer are associated with poor outcome. Breast Cancer Res Treat 2006;96: 91–5.
28. Jensen JD, Knoop A, Laenkholm AV, Grauslund M, Jensen MB, Santoni-Rugiu E, et al. PIK3CA mutations, PTEN, and pHER2 expression and impact on outcome in HER2-positive early-stage breast cancer patients treated with adjuvant chemotherapy and trastuzumab. Ann Oncol 2012;23: 2034–42.
29. Stemke-Hale K, Gonzalez-Angulo AM, Lluch A, Neve RM, Kuo WL, Davies M, et al. An integrative genomic and proteomic analysis of PIK3CA, PTEN, and AKT mutations in breast cancer. Cancer Res 2008; 68:6084–91.
30. Loi S, Michiels S, Lambrechts D, Fumagalli D, Claes B, Kellokumpu-Lehtinen PL, et al. Somatic mutation profiling and associations with prognosis and trastuzumab benefit in early breast cancer. J Natl Cancer Inst 2013;105:960–7.
31. Lopez-Knowles E, O'Toole SA, McNeil CM, Millar EK, Qiu MR, Crea P, et al. PI3K pathway activation in breast cancer is associated with the basal-like phenotype and cancer-specific mortality. Int J Cancer 2010; 126:1121–31.
32. Jin W, Wu L, Liang K, Liu B, Lu Y, Fan Z. Roles of the PI-3K and MEK pathways in Ras-mediated chemoresistance in breast cancer cells. Br J Cancer 2003;89:185–91.
33. Knuefermann C, Lu Y, Liu B, Jin W, Liang K, Wu L, et al. HER2/PI-3K/Akt activation leads to a multidrug resistance in human breast adenocarcinoma cells. Oncogene 2003;22:3205–12.
34. Liedtke C, Cardone L, Tordai A, Yan K, Gomez HL, Figureoa LJ, et al. PIK3CA-activating mutations and chemotherapy sensitivity in stage II-III breast cancer. Breast Cancer Res 2008;10:R27.
35. Majewski IJ, Nuciforo P, Mittempergher L, Bosma AJ, Eidtmann H, Holmes E, et al. PIK3CA mutations are associated with decreased benefit to neoadjuvant human epidermal growth factor receptor 2-targeted therapies in breast cancer. J Clin Oncol 2015 Jan 5. [Epub ahead of print].
36. Yao L, Liu Y, Li Z, Ouyang T, Li J, Wang T, et al. HER2 and response to anthracycline-based neoadjuvant chemotherapy in breast cancer. Ann Oncol 2011;22:1326–31.
37. Bear HD, Anderson S, Brown A, Smith R, Mamounas EP, Fisher B, et al. The effect on tumor response of adding sequential preoperative docetaxel to preoperative doxorubicin and cyclophosphamide: preliminary results from National Surgical Adjuvant Breast and Bowel Project Protocol B-27. J Clin Oncol 2003;21:4165–74.
38. Janku F, Wheler JJ, Naing A, Falchook GS, Hong DS, Stepanek VM, et al. PIK3CA mutation H1047R is associated with response to PI3K/AKT/mTOR signaling pathway inhibitors in early-phase clinical trials. Cancer Res 2013;73:276–84.
39. Janku F, Wheler JJ, Naing A, Stepanek VM, Falchook GS, Fu S, et al. PIK3CA mutations in advanced cancers: characteristics and outcomes. Oncotarget 2012;3:1566–75.

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40. Saal LH, Holm K, Maurer M, Memeo L, Su T, Wang X, et al. PIK3CA mutations correlate with hormone receptors, node metastasis, and ERBB2, and are mutually exclusive with PTEN loss in human breast carcinoma. Cancer Res 2005;65:2554–9.
41. Gonzalez-Angulo AM, Stemke-Hale K, Palla SL, Carey M, Agarwal R, Meric-Berstam F, et al. Androgen receptor levels and association with PIK3CA mutations and prognosis in breast cancer. Clin Cancer Res 2009;15: 2472–8.
42. Baselga J, Cortes J, Im SA, Clark E, Ross G, Kiermaier A, et al. Biomarker analyses in CLEOPATRA: a phase III, placebo-controlled study of pertuzumab in human epidermal growth factor receptor 2-positive, first-line metastatic breast cancer. J Clin Oncol 2014;32:3753–61.
43. Pogue-Geile KL, Song N, Jeong JH, Gavin PG, Kim SR, Blackmon NL, et al. Intrinsic subtypes, PIK3CA mutation, and the degree of benefit from adjuvant trastuzumab in the NSABP B-31 trial. J Clin Oncol 2015; 33:1340–7.

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Association of *PIK3CA* Mutation Status before and after Neoadjuvant Chemotherapy with Response to Chemotherapy in Women with Breast Cancer

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