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ORIGINAL ARTICLE – CANCER RESEARCH

Co-mutation of *TP53* and *PIK3CA* in residual disease after neoadjuvant chemotherapy is associated with poor survival in breast cancer

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Abstract

Purpose The prevalence and clinical relevance of *TP53* and *PIK3CA* mutations in pretreatment breast cancer have been previously reported. However, little is known regarding these mutations in residual tumor tissues after neoadjuvant chemotherapy. Here, we investigated the association between *TP53* and *PIK3CA* mutations in residual disease and survival of breast cancers.

Methods *TP53* and *PIK3CA* somatic mutations were examined in 353 post-neoadjuvant chemotherapy residual tumor tissues by Sanger sequencing. Survival curves of patients with *TP53* and *PIK3CA* mutations were compared using the Kaplan–Meier method.

Results Fifty-six (15.9%) of the 353 patients carried a *TP53* somatic mutation and 79 patients (22.4%) carried a *PIK3CA* somatic mutation. A total of 18 patients carried co-mutation of *TP53* and *PIK3CA*. Patients with somatic co-mutation were more likely to have high-grade tumors (35.3% vs. 10.6%, $P=0.010$), estrogen receptor-negative tumors (55.6% vs. 26.7%, $P=0.009$), progesterone receptor-negative tumors (61.1% vs. 30.5%, $P=0.008$) and triple-negative tumors (35.3% vs. 13.3%, $P=0.025$) compared with non-carriers. More importantly, co-mutation of *TP53* and *PIK3CA* carriers had a significantly worse disease-free survival (DFS) and distant disease-free survival (DDFS) than non-carriers (5-year DFS: 58.0% vs. 83.2%, $P<0.001$; 5-year DDFS: 70.3% vs. 86.4%, $P=0.024$). Furthermore, in multivariate regression analysis, *TP53* and *PIK3CA* co-mutation carriers showed a significantly worse DFS (adjusted hazard ratio = 3.70; 95% confidence interval, 1.79–7.63; $P<0.001$).

Conclusions Patients with somatic co-mutation of *TP53* and *PIK3CA* were associated with unfavorable survival compared with non-carriers. Co-mutation of *TP53* and *PIK3CA* could be used as a potential prognosis marker in post-neoadjuvant chemotherapy breast cancer patients.

Keywords Breast cancer · *TP53* · *PIK3CA* · Survival · Post-neoadjuvant chemotherapy

Introduction

Neoadjuvant chemotherapy has become part of the standard treatment for patients with locally advanced breast cancer (Penault-Llorca and Radosevic-Robin 2016). Approximately, 20% of patients with breast cancer who accepted neoadjuvant chemotherapy achieved pathological complete response (pCR) (Cortazar et al. 2014). However, patients with residual breast tumor after neoadjuvant chemotherapy are more common and these patients are associated with a higher risk of metastatic recurrence and unfavorable survival compared with patients who achieve pCR (Cortazar et al. 2014; Penault-Llorca and Radosevic-Robin 2016; Spring et al. 2017; Symmans et al. 2007). Therefore, it is important

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to investigate the molecular characteristics of post-neoadjuvant chemotherapy tumor tissues.

TP53 and *PIK3CA* are two of the most frequently mutated genes in breast cancer, each with a mutation frequency > 30% in pretreatment breast tumors (Cancer Genome Atlas 2012; Stephens et al. 2012). Most of the previous studies demonstrated that *TP53* somatic mutation in pretreatment tumor tissues was associated with poor survival of breast cancer patients (Berns et al. 2000; Falette et al. 1998; Olivier et al. 2006; Silwal-Pandit et al. 2014), whereas the role of *PIK3CA* somatic mutations in breast cancer survival remains controversial (Cizkova et al. 2012; Isakoff et al. 2005; Kalinsky et al. 2009; Li et al. 2006; Lopez-Knowles et al. 2010; Perez-Tenorio et al. 2007). However, the frequency and clinical relevance of somatic mutations in *TP53* and *PIK3CA* in unselected post-neoadjuvant chemotherapy breast tumors are largely unknown. Only one study reported that *TP53* somatic mutation frequently presented in residual tumor tissues of triple-negative breast cancer (Balko et al. 2014).

In vitro studies have indicated that *TP53* and *PIK3CA* co-mutated cells exhibit increased cancerous phenotypes (Croessmann et al. 2017). In vivo experiments also demonstrated that *TP53* and *PIK3CA* co-mutations cooperated in mouse mammary tumor formation (Adams et al. 2011). The somatic co-mutations of *TP53* and *PIK3CA* persisted in the residual tumor tissues after neoadjuvant chemotherapy may affect the survival in patients with breast cancer.

In this study, we investigated somatic mutations of *TP53* and *PIK3CA* genes in post-neoadjuvant chemotherapy residual tumor tissues of 353 primary breast cancer patients who received neoadjuvant chemotherapy; we further explored the associations between the *TP53* and *PIK3CA* somatic co-mutations and clinicopathological characteristics; and finally, we compared the survival of the *TP53* and *PIK3CA* co-mutation carriers, *TP53* mutation carriers, *PIK3CA* mutation carriers, and non-carriers in terms of disease-free survival (DFS) and distant disease-free survival (DDFS).

Materials and methods

Patients

This study included surgically resected tumor samples ($N = 446$) from patients diagnosed with operable primary (stage I–III) breast cancer and treated with neoadjuvant chemotherapy at the Breast Center, Peking University Cancer Hospital from 2003 to 2012. The tumor response after neoadjuvant chemotherapy was assessed by the Miller-Payne scoring system (Ogston et al. 2003). Among the 446 patients, 58 patients had a score of 5 (pCR) and 33 patients had a score of 4 (only small clusters or widely dispersed individual cells remain), and these patients were excluded. The remaining 355 patients, who had residual disease with a Miller-Payne score of 1, 2 or 3, were included in the study.

The patient age at diagnosis ranged from 25 to 73 years old, with a median of 50 years. Tumor size was defined as the maximum tumor diameter measured by ultrasound at the time of diagnosis. Follow-up data were available for all patients included in the study and the median length of follow-up was 75 months (range 7–160 months). During the follow-up period, 85 patients experienced a local or distant recurrence or died of the disease.

TP53 and *PIK3CA* mutation screening

Tumor DNA was extracted from fresh-frozen breast tumor tissues taken after neoadjuvant chemotherapy using Biotake DNA kit (Beijing, China) according to the manufacturer's instructions. Mutational analysis of *TP53* and *PIK3CA* was performed using a set of primer pairs that covered exons 3–11 of *TP53* and exon 9 and exon 20 of *PIK3CA* (Table S1). All fragments were sequenced using the BigDye Terminator Cycle Sequencing Kit and ABI3730 automated sequencer (Applied Bio-systems). Each mutation was confirmed by independent PCRs and Sanger sequencing in duplicate. *TP53* and *PIK3CA* mutations were successfully identified in 353 of 355 patients (99.4%).

Pathologic assessment

Estrogen receptor (ER), progesterone receptor (PR) and human epidermal growth factor receptor 2 (HER2) statuses were determined in core-needle breast tumor tissue obtained before initiation of neoadjuvant chemotherapy. ER or PR was positive when ≥ 1% of the tumor cells showed positive nuclear staining. HER2 positivity was defined as a score of 3 + by immunohistochemical staining or HER2 gene amplification by fluorescence *in situ* hybridization. pCR was defined as no invasive breast cancer cells in the breast or lymph node after completion of neoadjuvant chemotherapy.

Neoadjuvant chemotherapy

All 353 patients received neoadjuvant chemotherapy, and 95% of the patients received four to eight cycles. Patients were categorized into four treatment subgroups as follows:

1. A total of 216 patients received anthracycline/cyclophosphamide-based neoadjuvant regimens. Of these, 63 patients received CTF (5-fluorouracil, pirarubicin and cyclophosphamide) regimen; 130 patients received FEC (5-fluorouracil, epirubicin and cyclophosphamide); the remaining 23 patients received CAF (5-fluorouracil, doxorubicin and cyclophosphamide) or AC (doxorubicin and cyclophosphamide).

2. A total of 110 patients received anthracycline–taxane-based regimens. Of these, 69 patients received two cycles of anthracycline regimens followed by four cycles of paclitaxel alone or docetaxel plus cyclophosphamide, and 41 patients received two cycles of anthracycline regimens followed by paclitaxel plus carboplatin.
3. A total of 27 patients received taxane-based regimens.

Details of these neoadjuvant chemotherapy treatments are described in our previous study (Wang et al. 2016). Among the 77 patients with HER2-positive tumors, 9 women were treated with neoadjuvant trastuzumab in combination with one of the above-described regimens.

Statistical analyses

Associations between *TP53* and/or *PIK3CA* mutations and clinicopathological characteristics were examined using the Chi-square test or the Fisher exact test. 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. 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 the statistical significance of differences was tested using the log-rank test. Cox proportional hazard regression models were used to test the prognostic role of *TP53* and/or *PIK3CA* mutation status [hazard ratio (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 21.0 software (Chicago, IL, USA).

Results

Frequency of *TP53* and/or *PIK3CA* mutations

TP53 and *PIK3CA* somatic mutations were determined in the post-neoadjuvant chemotherapy tumor tissue of 353 breast cancer patients. The clinicopathological characteristics of these patients are presented in Table 1. A total of 56 patients (15.9%, 56/353) carried *TP53* somatic mutation and 79 patients (22.4%, 79/353) carried *PIK3CA* somatic mutation. 235 patients (66.6%, 235/353) were non-carriers. Eighteen patients (5.2%, 18/353) had both *TP53* and *PIK3CA* somatic mutations simultaneously (Table S2).

Among the 56 *TP53* mutations, 6 were nonsense mutations, 12 were frameshift mutations, 37 were missense mutation and one was a non-frameshift deletion. The distribution of the *TP53* mutations was non-uniform across the gene. A total of 46 of the 56 mutations (82%) were located in exons 5–8, which span the DNA-binding domain of the protein. Six *TP53* mutations (p.P12fs*25, p.P141_L145>W, p.M160fs*20, p.S261fs*3, p.R290fs*61, and p.E298fs*8) were novel and have not been reported in databases or the previous literature.

Among the 79 *PIK3CA* mutations, 28 mutations were located in the helical domain (exon 9) and 51 mutations clustered in the kinase domain (exon 20). All the *PIK3CA* mutations identified in this study were missense mutations and have been previously reported.

Table 1 Clinical characteristics in the 353 breast cancer patients

Study characteristic	No.	%
Age at diagnosis, years		
Mean ± SD	48.7 ± 9.4	
Median	50	
Range	25–73	
≤ 50	186	52.7
> 50	167	47.3
Tumor size		
≤ 2 cm	86	24.4
> 2 cm	267	75.6
Histology		
Ductal	334	94.6
Lobular	13	3.7
Medullary	1	0.3
Mucinous	2	0.6
Other	3	0.8
Grade		
I	34	9.6
II	254	72.0
III	40	11.3
Unknown	25	7.1
ER status		
Negative	95	26.9
Positive	258	73.1
PR status		
Negative	112	31.7
Positive	241	68.3
HER2 status		
Negative	244	69.1
Positive	75	21.2
Unknown	34	9.6
Lymph nodes status		
Negative	120	34.0
Positive	217	61.6
Unknown	16	4.5

ER estrogen receptor, *PR* progesterone receptor, *HER2* human epidermal growth factor receptor 2

TP53 and/or *PIK3CA* mutation and tumor characteristics

Detailed clinical information of the 18 patients that carried somatic co-mutation of *TP53* and *PIK3CA* is presented in Table S3. These co-mutation carriers were more likely to have high-grade tumors (35.3% vs. 10.6%, $P=0.010$), ER-negative tumors (55.6% vs. 26.7%, $P=0.009$), PR-negative tumors (61.1% vs. 30.5%, $P=0.008$) and triple-negative tumors (35.3% vs. 13.3%, $P=0.025$) compared with non-carriers (Table 2). No significant differences in age at diagnosis, histology, tumor size, HER2 status and lymph node status were observed between co-mutation carriers and non-carriers.

Table 2 Association of patient and tumor characteristics with mutation status

Study characteristic	No.	Co-mutation ($n=18$)		*TP53* only ($n=38$)		*PIK3CA* only ($n=61$)		Non-carriers ($n=236$)		*P*1	*P*2	*P*3
		No.	%	No.	%	No.	%	No.	%			
Age at diagnosis, years												
Mean ± SD	48.7 ± 9.4	48.2 ± 11.1		48.3 ± 9.8		48.5 ± 9.4		48.9 ± 9.3				
≤ 50	186	10	55.6	22	57.9	29	47.5	127	53.8	0.89	0.64	0.38
> 50	167	8	44.4	16	42.1	32	52.5	109	46.2			
Tumor size												
≤ 2 cm	86	3	16.7	9	23.7	14	23.0	60	25.4	0.57	0.82	0.69
> 2 cm	267	15	83.3	29	76.3	47	77.0	176	74.6			
Histology												
Ductal	334	17	94.4	37	97.4	56	93.3	224	95.7	0.57	1.00	0.50
Lobular	13	0	0.0	0	0.0	4	6.7	9	3.8			
Medullary	1	0	0.0	1	2.6	0	0.0	0	0.0			
Mucinous	2	1	5.6	0	0.0	0	0.0	1	0.4			
Other	3	0		0		1		2				
Grade												
I	35	0	0.0	1	2.7	9	16.1	23	10.6	0.010	0.10	0.23
II	262	11	74.7	28	75.7	44	78.6	171	78.8			
III	43	6	35.3	8	21.6	3	5.4	23	10.6			
Unknown	27	1		1		5		19				
ER status										0.009	0.010	0.005
Negative	100	10	55.6	18	47.4	6	9.8	63	26.7			
Positive	267	8	44.4	20	52.6	55	90.2	173	73.3			
PR status										0.008	0.003	0.028
Negative	121	11	61.1	21	55.3	10	16.4	72	30.5			
Positive	246	7	38.9	17	44.7	51	83.6	164	69.5			
HER2 status										0.23	0.018	0.11
Negative	255	11	64.7	21	60.0	45	88.2	171	78.4			
Positive	77	6	35.3	14	40.0	6	11.8	47	21.6			
Unknown	35	1		3		10		18				
TNBC										0.025	0.18	0.16
Yes	50	6	35.3	8	21.6	4	6.7	31	13.3			
No	310	11	46.7	29	78.4	56	93.3	202	86.7			
Unknown	7	1		1		1		3				
Lymph nodes status										0.94	0.75	0.45
Negative	120	6	35.3	13	37.1	23	39.7	78	34.4			
Positive	229	11	46.7	22	62.9	35	60.3	149	65.6			
Unknown	18	1		3		3		9				

ER estrogen receptor, *PR* progesterone receptor, *HER2* human epidermal growth factor receptor 2, *TNBC* triple-negative breast cancer, *P1 TP53* and *PIK3CA* co-mutation carriers vs. non-carriers, *P2 TP53* only mutation carriers vs. non-carriers, *P3 PIK3CA* only mutation carriers vs. non-carriers

Analysis of the single mutation carriers revealed that only *TP53* mutation carriers were significantly more likely to have ER-negative, PR-negative and HER2-positive tumors compared with non-carriers (Table 2). Only *PIK3CA* mutation carriers were also more likely to have ER-positive and PR-positive tumors than non-carriers (Table 2).

Survival

We next analyzed survival in the 353 breast cancer patients. The 5-year DFS rates in *TP53* and *PIK3CA* co-mutation carriers and non-carriers were 58.0% and 83.2% ($P < 0.001$), respectively, and the 5-year DDFS rates in co-mutation carriers and non-carriers were 70.3% and 86.4% ($P = 0.024$), respectively. *TP53* and *PIK3CA* co-mutation carriers exhibited a significantly worse DFS compared with non-carriers (unadjusted HR = 3.47; 95% CI 1.78–6.76; $P < 0.001$) (Table 3; Fig. 1). Although *TP53* only mutation carriers showed a slightly poor DFS and DDFS compared with non-carriers, the differences did not reach significance (DFS: unadjusted HR = 1.75; 95% CI 0.96–3.19; $P = 0.07$; and DDFS: unadjusted HR = 1.44; 95% CI 0.72–2.90; $P = 0.30$). There were no significant differences in DFS and DDFS between the *PIK3CA* only mutation carriers and non-carriers (DFS: unadjusted HR, 1.03; 95% CI 0.55–1.94; $P = 0.93$; and DDFS: unadjusted HR, 1.21; 95% CI 0.63–2.30; $P = 0.57$).

Table 3 Univariate analyses of disease-free survival and distant disease-free survival in the 353 breast cancer patients

variable	DFS		DDFS	
	HR (95% CI)	*P*	HR (95% CI)	*P*
Age (years)				
≤ 50	1.00		1.00	
> 50	1.00 (0.65–1.54)	1.00	0.96 (0.60–1.54)	0.87
Tumor size				
≤ 2 cm	1.00		1.00	
> 2 cm	1.68 (0.94–2.98)	0.08	1.81 (0.95–3.45)	0.07
Lymph nodes status				
Negative	1.00		1.00	
Positive	2.98 (1.67–5.30)	< 0.001	3.15 (1.65–6.01)	< 0.001
Grade				
I + II	1.00		1.00	
III	1.11 (0.59–2.12)	0.74	1.08 (0.53–2.20)	0.83
ER status				
Negative	1.00		1.00	
Positive	0.77 (0.48–1.22)	0.27	1.15 (0.66–1.99)	0.62
PR status				
Negative	1.00		1.00	
Positive	0.80 (0.51–1.26)	0.33	0.92 (0.56–1.53)	0.76
HER2 status				
Negative	1.00		1.00	
Positive	1.89 (1.18–3.02)	0.008	1.14 (0.66–1.99)	0.64
Surgery type				
Mastectomy	1.00		1.00	
BCS	1.35 (0.82–2.22)	0.23	1.67 (0.94–2.97)	0.08
Treatments				
C	1.00		1.00	
C + E	0.76 (0.47–1.21)	0.27	0.99 (0.57–1.74)	0.98
Mutation				
Non-carriers	1.00		1.00	
TP53 only	1.75 (0.96–3.19)	0.07	1.44 (0.72–2.90)	0.30
PIK3CA only	1.03 (0.55–1.94)	0.93	1.21 (0.63–2.30)	0.57
Co-mutation	3.47 (1.78–6.76)	< 0.001	2.24 (0.99–5.11)	0.05

DFS disease-free survival, *DDFS* distant disease-free survival, *HR* hazard ratio, *CI* confidence interval, *ER* estrogen receptor, *PR* progesterone receptor, *HER2* human epidermal growth factor receptor 2, *BCS* breast-conserving surgery, *C* chemotherapy, *E* endocrine therapy

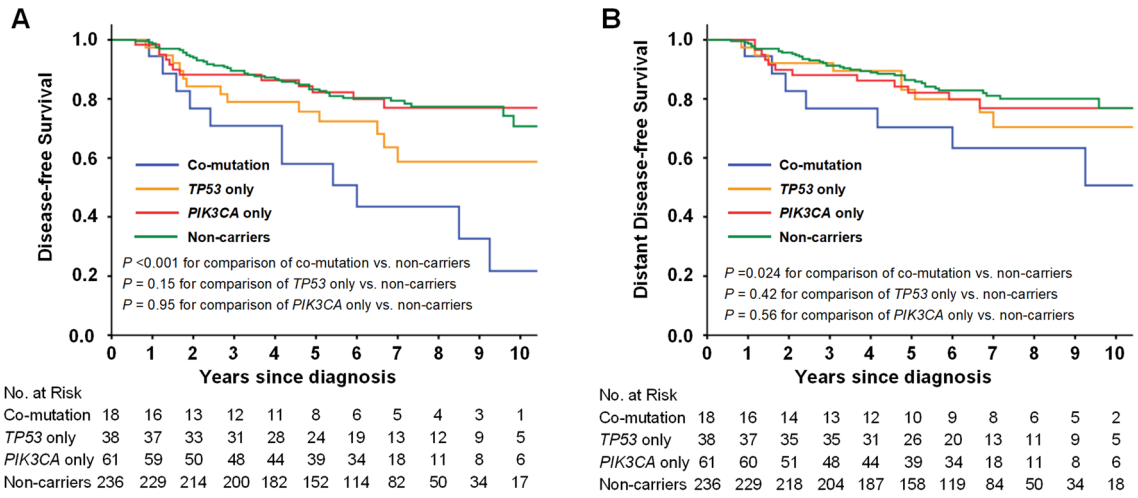

Fig. 1 Survival analyses by Kaplan–Meier according to *TP53* and *PIK3CA* mutation status in the 353 post-neoadjuvant chemotherapy breast cancer patients. **a, b** Disease-free survival and distant disease-free survival according to mutation status, respectively

Furthermore, in multivariate regression analysis, *TP53* and *PIK3CA* co-mutation carriers showed a significantly worse DFS (adjusted HR = 3.70; 95% CI 1.79–7.63; $P < 0.001$) and a trend of worse DDFS (adjusted HR = 2.12; 95% CI 0.86–5.24; $P = 0.10$) after adjustment for age, tumor size, lymph node, tumor grade, ER status, PR status, HER2 status and treatment (Table 4). *TP53* only mutation and *PIK3CA* only mutation failed to demonstrate an independent effect on DFS or DDFS.

Discussion

Here, we investigated the association between *TP53* and *PIK3CA* somatic co-mutation and survival in 353 post-neoadjuvant chemotherapy Chinese breast cancer patients. We found that patients with *TP53* and *PIK3CA* co-mutations exhibited a poorer survival compared with non-carriers.

In the current cohort, the somatic mutation frequencies of *TP53* and *PIK3CA* in post-neoadjuvant chemotherapy breast tumor tissues were 15.9% and 22.4%, respectively. To the best of our knowledge, no study has previously reported the prevalence of *TP53* or *PIK3CA* somatic mutations in unselected post-neoadjuvant chemotherapy breast tumors. In our previous study, the *TP53* and *PIK3CA* somatic mutations were detected in core-needle biopsy samples that were taken before neoadjuvant chemotherapy; the mutation frequency of *TP53* and *PIK3CA* in pre-neoadjuvant chemotherapy tumor tissues was 41.0% and 28.3%, respectively (Wang et al. 2016; Yuan et al. 2015). These indicated that the mutation rate of both genes may be decreased after neoadjuvant chemotherapy. Unfortunately, there were no matching pre-neoadjuvant chemotherapy tumor tissues for sequencing in this cohort. Jiang et al. reported that the frequencies of *TP53* and *PIK3CA* somatic mutations were lower in post-neoadjuvant chemotherapy tumor tissues compared with in paired pre-neoadjuvant chemotherapy samples (Jiang et al. 2014). These results suggested that *TP53* and *PIK3CA* mutations in some breast cancers might be lost after neoadjuvant chemotherapy (Kim et al. 2018; Yates et al. 2015). This possibility needs to be further explored in larger paired samples.

The associations between the somatic co-mutation of *TP53* and *PIK3CA* and clinicopathological characteristics in breast cancer were previously not well known. In this cohort, the co-mutation carriers were more likely to have high-grade tumors, ER-negative tumors, PR-negative tumors and triple-negative tumors compared with non-carriers. These results suggested that breast cancer patients carrying a *TP53* and *PIK3CA* co-mutation may have a more aggressive phenotype compared with non-carriers. Our data showed that there was no difference in survival between *PIK3CA* only mutation carriers and non-carriers among the 353 breast cancer patients. The prognosis of patients with *TP53* somatic mutation was slightly worse than those without *TP53* or *PIK3CA* mutations, but the difference did not reach statistical significance. However, *TP53* and *PIK3CA* co-mutation carriers showed a significantly worse DFS and DDFS compared with non-carriers. Moreover, co-mutation carriers had a significantly worse DFS and a tendency towards worse DDFS than non-carriers in multivariate analysis. In mouse models, co-mutation of *TP53* and *PIK3CA* showed a cooperation in mammary tumor formation (Adams et al. 2011) and induced more aggressive mammary tumors (Thakur and Ray 2016; Van Keymeulen et al. 2015). Previous reports studies mainly investigated the association between the mutation status of *TP53* or *PIK3CA* before neoadjuvant chemotherapy and survival in breast cancer patients, but the mutation status may be changed after neoadjuvant chemotherapy (Jiang et al.

Table 4 Multivariate analyses of disease-free survival and distant disease-free survival in the 353 breast cancer patients

variable	DFS		DDFS	
	HR (95% CI)	*P*	HR (95% CI)	*P*
Age (years)				
≤50	1.00		1.00	
>50	0.85 (0.52–1.38)	0.51	0.86 (0.51–1.47)	0.59
Tumor size				
≤2 cm	1.00		1.00	
>2 cm	1.55 (0.82–2.96)	0.18	1.65 (0.80–3.41)	0.17
Lymph nodes status				
Negative	1.00		1.00	
Positive	3.33 (1.78–6.24)	<0.001	2.87 (1.46–5.62)	0.002
Grade				
I+II	1.00		1.00	
III	1.11 (0.55–2.24)	0.77	1.24 (0.57–2.71)	0.56
ER status				
Negative	1.00		1.00	
Positive	0.49 (0.16–1.51)	0.22	1.37 (0.37–5.02)	0.64
PR status				
Negative	1.00		1.00	
Positive	1.18 (0.56–2.51)	0.66	0.70 (0.31–1.55)	0.38
HER2 status				
Negative	1.00		1.00	
Positive	1.88 (1.09–3.23)	0.023	1.06 (0.56–2.03)	0.82
Surgery type				
Mastectomy	1.00		1.00	
BCS	0.94 (0.53–1.67)	0.83	0.72 (0.38–1.38)	0.33
Treatments				
C	1.00		1.00	
C+E	1.69 (0.53–5.46)	0.38	1.09 (0.29–4.10)	0.90
Mutation				
Non-carriers	1.00		1.00	
TP53 only	1.67 (0.84–3.34)	0.14	1.38 (0.62–3.04)	0.43
PIK3CA only	1.09 (0.52–2.28)	0.82	1.17 (0.56–2.45)	0.68
Co-mutation	3.70 (1.79–7.63)	<0.001	2.12 (0.86–5.24)	0.10

DFS disease free survival, *DDFS* distant disease-free survival, *HR* hazard ratio, *CI* confidence interval, *ER* estrogen receptor, *PR* progesterone receptor, *HER2*, human epidermal growth factor receptor 2, *BCS* breast-conserving surgery, *C* chemotherapy, *E* endocrine therapy

2014). For patients who did not achieve pCR after neoadjuvant chemotherapy, one advantage of the current study was that we evaluated the prognostic value of *TP53* or *PIK3CA* mutation in the residual tumor, and patients with remained *TP53* or *PIK3CA* mutation after neoadjuvant chemotherapy may be the potentially candidates for selecting other post-operation chemotherapy regimens or a targeted therapy in clinical trials.

One of the limitations of this study is the lack of matched pre-neoadjuvant chemotherapy tumor tissue for mutation analysis. In addition, the number of co-mutation carriers was relatively small, and therefore, interpretation of the results should be cautious. Furthermore, we cannot figure out whether *TP53 and PIK3CA* mutations occur in the same tumor cells or the mutations present in distinct cells in the current study; this issue may be addressed by a single cell sequencing in a future study.

In conclusion, here we found that *TP53* and *PIK3CA* co-mutation rate was 5.2% in residual breast cancer after neoadjuvant chemotherapy in the current cohort. The co-mutation is associated with high-grade tumor, ER-negative tumors, PR-negative tumors, and triple-negative tumors, and more importantly, it is associated with a poorer survival. Nevertheless, independent studies with large sample sizes or matched samples are warranted to confirm our current findings.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval The study was conducted in accordance with Helsinki Declaration, and was approved by the Research Ethics Committee of Peking University Cancer Hospital.

Informed consent Written informed consent was obtained from all participants.

References

Adams JR, Xu K, Liu JC et al (2011) Cooperation between Pik3ca and p53 mutations in mouse mammary tumor formation. Cancer Res 71:2706–2717. https://doi.org/10.1158/0008-5472.CAN-10-0738

Balko JM, Giltnane JM, Wang K et al (2014) Molecular profiling of the residual disease of triple-negative breast cancers after neoadjuvant chemotherapy identifies actionable therapeutic targets. Cancer Discov 4:232–245. https://doi.org/10.1158/2159-8290.CD-13-0286

Berns EM, Foekens JA, Vossen R et al (2000) Complete sequencing of TP53 predicts poor response to systemic therapy of advanced breast cancer. Cancer Res 60:2155–2162

Cancer Genome Atlas N (2012) Comprehensive molecular portraits of human breast tumours. Nature 490:61–70. https://doi.org/10.1038/nature11412

Cizkova M, Susini A, Vacher S et al (2012) PIK3CA mutation impact on survival in breast cancer patients and in ERalpha, PR and ERBB2-based subgroups. Breast Cancer Res 14:R28. https://doi.org/10.1186/bcr3113

Cortazar P, Zhang L, Untch M et al (2014) Pathological complete response and long-term clinical benefit in breast cancer: the CTNeoBC pooled analysis. Lancet 384:164–172. https://doi.org/10.1016/S0140-6736(13)62422-8

Croessmann S, Wong HY, Zabransky DJ et al (2017) PIK3CA mutations and TP53 alterations cooperate to increase cancerous

phenotypes and tumor heterogeneity. Breast Cancer Res Treat 162:451–464. https://doi.org/10.1007/s10549-017-4147-2

Falette N, Paperin MP, Treilleux I et al (1998) Prognostic value of P53 gene mutations in a large series of node-negative breast cancer patients. Cancer Res 58:1451–1455

Isakoff SJ, Engelman JA, Irie HY et al (2005) Breast cancer-associated PIK3CA mutations are oncogenic in mammary epithelial cells. Cancer Res 65:10992–11000. https://doi.org/10.1158/0008-5472.CAN-05-2612

Jiang YZ, Yu KD, Bao J et al (2014) Favorable prognostic impact in loss of TP53 and PIK3CA mutations after neoadjuvant chemotherapy in breast cancer. Cancer Res 74:3399–3407. https://doi.org/10.1158/0008-5472.CAN-14-0092

Kalinsky K, Jacks LM, Heguy A et al (2009) PIK3CA mutation associates with improved outcome in breast cancer. Clin Cancer Res 15:5049–5059. https://doi.org/10.1158/1078-0432.CCR-09-0632

Kim C, Gao R, Sei E et al (2018) Chemoresistance evolution in triple-negative breast cancer delineated by single-cell sequencing. Cell 173:879–893 e813. https://doi.org/10.1016/j.cell.2018.03.041

Li SY, Rong M, Grieu F et al (2006) PIK3CA mutations in breast cancer are associated with poor outcome. Breast Cancer Res Treat 96:91–95. https://doi.org/10.1007/s10549-005-9048-0

Lopez-Knowles E, O'Toole SA, McNeil CM et al (2010) PI3K pathway activation in breast cancer is associated with the basal-like phenotype and cancer-specific mortality. Int J Cancer 126:1121–1131. https://doi.org/10.1002/ijc.24831

Ogston KN, Miller ID, Payne S et al (2003) A new histological grading system to assess response of breast cancers to primary chemotherapy: prognostic significance and survival. Breast 12:320–327

Olivier M, Langerod A, Carrieri P et al (2006) The clinical value of somatic TP53 gene mutations in 1,794 patients with breast cancer. Clin Cancer Res 12:1157–1167. https://doi.org/10.1158/1078-0432.CCR-05-1029

Penault-Llorca F, Radosevic-Robin N (2016) Biomarkers of residual disease after neoadjuvant therapy for breast cancer. Nat Rev Clin Oncol 13:487–503. https://doi.org/10.1038/nrclinonc.2016.1

Perez-Tenorio G, Alkhori L, Olsson B et al (2007) PIK3CA mutations and PTEN loss correlate with similar prognostic factors and are not mutually exclusive in breast cancer. Clin Cancer Res 13:3577–3584. https://doi.org/10.1158/1078-0432.CCR-06-1609

Silwal-Pandit L, Vollan HK, Chin SF et al (2014) TP53 mutation spectrum in breast cancer is subtype specific and has distinct prognostic relevance. Clin Cancer Res 20:3569–3580. https://doi.org/10.1158/1078-0432.CCR-13-2943

Spring L, Greenup R, Niemierko A et al (2017) Pathologic complete response after neoadjuvant chemotherapy and long-term outcomes among young women with breast cancer. J Natl Compr Canc Netw 15:1216–1223. https://doi.org/10.6004/jnccn.2017.0158

Stephens PJ, Tarpey PS, Davies H et al (2012) The landscape of cancer genes and mutational processes in breast cancer. Nature 486:400–404. https://doi.org/10.1038/nature11017

Symmans WF, Peintinger F, Hatzis C et al (2007) Measurement of residual breast cancer burden to predict survival after neoadjuvant chemotherapy. J Clin Oncol 25:4414–4422. https://doi.org/10.1200/JCO.2007.10.6823

Thakur B, Ray P (2016) p53 Loses grip on PIK3CA expression leading to enhanced cell survival during platinum resistance. Mol Oncol 10:1283–1295. https://doi.org/10.1016/j.molonc.2016.06.006

Van Keymeulen A, Lee MY, Ousset M et al (2015) Reactivation of multipotency by oncogenic PIK3CA induces breast tumour heterogeneity. Nature 525:119–123. https://doi.org/10.1038/nature14665

Wang Y, Xu Y, Chen J et al (2016) TP53 mutations are associated with higher rates of pathologic complete response to anthracycline/cyclophosphamide-based neoadjuvant chemotherapy in operable primary breast cancer. Int J Cancer 138:489–496. https://doi.org/10.1002/ijc.29715

Yates LR, Gerstung M, Knappskog S et al (2015) Subclonal diversification of primary breast cancer revealed by multiregion sequencing. Nat Med 21:751–759. https://doi.org/10.1038/nm.3886

Yuan H, Chen J, Liu Y et al (2015) Association of PIK3CA mutation status before and after neoadjuvant chemotherapy with response to chemotherapy in women with breast cancer. Clin Cancer Res 21:4365–4372. https://doi.org/10.1158/1078-0432.CCR-14-3354