Prognostic role of PIK3CA mutations and their association with hormone receptor expression in breast cancer: a meta-analysis

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The phosphatidylinositol-4, 5-bisphosphate 3-kinase, catalytic subunit alpha (PIK3CA) gene is frequently mutated in breast cancer (BCa). Sex hormone receptors (HRs), including estrogen receptor (ER) and progesterone receptor (PR) play pivotal roles in BCa. In this study, we evaluated the association between PIK3CA mutations and ER/PR expression and the prognostic role of PIK3CA mutations in BCa patients, and in particular, HR-positive BCa. Thirty-two studies involving 5719 cases of BCa obtained from database searches were examined. PIK3CA gene mutations correlated significantly with ER/PR expression ($p < 0.00001$) and relapse-free survival (RFS) (hazard ratio [HR] 0.76, 95% confidence interval [CI] 0.59–0.98, $p = 0.03$) but not overall survival (OS) (HR 1.14, 95%CI 0.72–1.82, $p = 0.57$) in unsorted BCa patients. PIK3CA mutations were not associated with OS (HR 1.06, 95%CI 0.67–1.67, $p = 0.81$) or RFS (HR 0.86, 95%CI 0.53–1.40, $p = 0.55$) in HR-positive BCa patients. In conclusion, PIK3CA mutations were significantly related to ER/PR expression and RFS in unsorted BCa patients. However, the clinical implications of PIK3CA mutations may vary according to different mutant exons. And PIK3CA mutations alone may have limited prognostic value for HR-positive BCa patients.

Breast cancer (BCa) is one of the most common cancers among women, with more than 1,300,000 new cases and about 450,000 deaths reported each year worldwide. This highly heterogeneous disease is divided into subgroups on the basis of molecular signatures, clinicopathologic features, and responses to therapy. Hormone receptors (HRs), including estrogen receptors (ERs) and progesterone receptors (PRs) are the most important markers of BCa. Most BCa cases are HR-positive (HR$^+$), and ER-positive (ER$^+$) BCa accounts for up to 80% of BCa cases among women 45 years and older. Endocrine therapy is regarded as the cornerstone of ER$^+$ BCa treatment. However, because of de novo or acquired resistance to endocrine therapy, prognosis is still poor for many ER$^+$ BCa patients. Therefore, finding new effective treatment methods for ER$^+$ BCa patients resistant to endocrine therapy is imperative.

After the TP53 gene, the phosphatidylinositol-4,5-bisphosphate 3-kinase, catalytic subunit alpha (PIK3CA) gene is the most frequently mutated gene in BCa. Phosphatidylinositol 3-kinase (PI3K) is composed of an 85-kD (p85) and a 110-kD (p110) subunit. When coupled to activated tyrosine kinases via p85 (the adaptor subunit), p110 (the catalytic subunit) phosphorylates the 3-hydroxy group of inositol phospholipids. Gain-of-function mutations in PIK3CA have been found in different types of cancers including BCa. The mutations result in PI3K activation independent of upstream signaling and constitutive activation of the downstream AKT pathway and may contribute to oncogenesis. The frequency of PIK3CA mutations in BCa cases ranges from 16.4 to 45%. There are 3 mutation “hotspots” in the PIK3CA gene: E542K, E545K at exon 9 (helix domain) and H1047R at exon 20 (kinase domain). The 3 hotspots represent almost 80% of PIK3CA mutations and lead to constitutive PI3K activity by different mechanisms.

Aberrant activation of the PI3K pathway is thought to contribute significantly to endocrine therapy resistance in patients with ER$^+$ BCa. There is evidence showing that endocrine therapy combined with p110 inhibitors is an effective treatment for ER$^+$ BCa cases, including those with PIK3CA mutations. The synthetic lethal interaction is a promising approach that needs further studies. Testing of several p110 inhibitors is underway in phase II clinical trials. Therefore, evaluation of the relationship between HRs and PIK3CA mutations in BCa is neces-
sary. It is also of great clinical interest to determine whether PIK3CA mutations are prognostic factors in HR+ BCa patients.

Results

Search results and description of eligible studies. A total of 1903 potentially relevant citations were retrieved. After exclusion of non-human studies, reviews, and duplicates, two authors independently perused the titles and abstracts of the articles. After screenings, 68 articles were chosen for further full-text review. Ultimately, 32 eligible studies were included in our meta-analysis5-39 (Figure 1).

The 32 eligible studies were published from 2004 to 2014 and involved 5719 cases. Data from the studies were grouped as follows: group A evaluated the relationship between PIK3CA mutations and ER (26 studies) or PR (20 studies) expression in BCa patients, group B (12 studies) and group C (8 studies) evaluated the relationship between PIK3CA mutations and the outcomes of all BCa patients and HR+ BCa patients, respectively. In the 32 selected studies, the percentage of patients with PIK3CA mutations ranged from 7.1% to 44.6%, and the percentage of ER+ patients ranged from 48.1% to 84.0%. For PR, the percentage ranged from 41.4% to 64.8%. In the B and C groups, the median follow-up time ranged from 50 to 153.6 months.

ER and PR expression and PIK3CA gene mutations in BCa patients. The relationship between PIK3CA gene mutations and ER expression was investigated in 4754 patients from 26 selected studies (Group A, the ER arm) using a fixed-effect model (Table 1). There was a significant association between PIK3CA gene mutations and ER expression in the patients in this group (odds ratio [OR] 1.92, 95%CI 1.65–2.23; P < 0.00001; Figure 2). Then we performed a separate analysis for PR expression in 3507 patients from 20 studies (Group A, the PR arm) using a fixed-effect model (Table 1), and found that PR expression was also significantly associated with PIK3CA mutations (OR 1.88, 95% CI 1.61–2.20; P < 0.00001) (Figure 3). Direct sequencing was the most frequently used method for detecting mutations in the selected studies. We introduced subgroups and found that direct sequencing and the other mutation detection methods produced similar results (p = 0.13).

PIK3CA gene mutations and prognosis in all BCa patients. Analyses were conducted to evaluate the relationship between PIK3CA gene mutations and prognosis as defined by overall survival (OS) and relapse-free survival (RFS) in all BCa patients (group B) (Table 2). Because of significant heterogeneity among the group B studies for OS (P = 0.008; I2 = 66%), a random-effect model was used to assess OS correlations. However, because there
| First author         | Year of publication | Country   | Design | Mean age(years) | No. of ER positive patients (%) | No. of PR positive patients (%) | No. of PIK3CA mutant patients (%) | Sequenced PIK3CA analysis methods |
|---------------------|---------------------|-----------|--------|-----------------|-------------------------------|-------------------------------|-----------------------------------|----------------------------------|
| Bachman KE          | 2004                | USA       | HB     | NR              | 28 (68.3)                     | 23 (57.5)                     | 9 (22.0)                          | exon 1 and 20 DS                  |
| Benvenuti S         | 2008                | Italy     | HB     | NR              | 95 (76.0)                     | 79 (64.8)                     | 28 (16.0)                         | exon 9 and 20 DS                  |
| Bozhanov SS         | 2010                | Bulgaria  | HB     | NR              | 81 (55.9)                     | 81 (56.3)                     | 45 (31.3)                         | exon 9 and 20 DS                  |
| Cizkova M           | 2012                | France    | HB     | 61.6 (31–91)    | 335 (74.1)                    | 258 (57.1)                    | 151 (33.4)                        | exon 9 and 20 DS                  |
| Dunlap J            | 2010                | USA       | HB     | NR              | 66 (81.5)                     | 42 (51.9)                     | 12 (14.8)                         | exon 9, 7 and 20 DS               |
| Li H                | 2010                | China     | HB     | 51 (33-80)      | 137 (83.0)                    | 100 (60.6)                    | 43 (26.1)                         | exon 9 and 20 DS                  |
| Liang X             | 2006                | Singapore | HB     | NR              | 37 (48.1)                     | 41 (53.2)                     | 31 (38.8)                         | exon 9 and 20 DS                  |
| Liedtke C           | 2008                | USA       | HB (stage II–III) | 51 (28–73) | 78 (55.7) | 58 (41.4) | 23 (16.4) | exon 9 and 20 DS |
| Lin CH              | 2011                | China(Taiwan) | HB     | NR (less than 35 y) | 81 (69.8) | 67 (57.8) | 22 (19.0) | exon 9 and 20 DS |
| Mangone FR          | 2012                | Brazil    | HB     | 55 (26–85)      | 53 (61.6)                     | 37 (46.3)                     | 22 (30.6)                         | exon 9 and 20 DS                  |
| Maruyama N          | 2007                | Japan     | HB     | NR              | 124 (66.0)                    | 114 (61.0)                    | 54 (28.7)                         | exon 1, 2, 4, 7, 9, 13, 18, and 20 DS |
| Michelucci A        | 2009                | Italy     | HB     | 43.5 (32–61)    | 98 (76.0)                     | 88 (61.5)                     | 63 (35.8)                         | exon 9 and 20 DS                  |
| Saal LH             | 2005                | USA       | HB     | 59 (24–89)      | 162 (55.5)                    | 142 (51.4)                    | 77 (26.4)                         | exon 1, 2, 4, 5, 7, 9, 12, 13, 18, 20 DS |
| Sanchez CG          | 2011                | USA       | HB     | 53.4 (32–80)    | 32 (62.7)                     | NR                            | 16 (31.4)                         | exon 9 and 20 (HS)                |
| Barbareschi M       | 2007                | Italy     | HB     | 62 (17–89)      | 137 (84.0)                    | 96 (60.1)                     | 45 (27.6)                         | exon 9 and 20 SSCP + DS           |
| Butitta F           | 2006                | Italy     | HB     | 57.2*           | 124 (68.9)                    | 106 (58.9)                    | 46 (25.6)                         | exon 1–20 SSCP + DS               |
| Campbell IG         | 2004                | Australia | HB     | NR              | 32 (62.7)                     | NR                            | 22 (43.1)                         | exon 1–20 SSCP + DHPLC            |
| Dupont Jensen J     | 2011                | Denmark   | HB     | 57 (32–87)      | 78 (77.2)                     | NR                            | 45 (44.6)                         | exon 9 and 20 (HS) SNaPshot/DxS   |
| Harlé A             | 2013                | France    | HB     | NR              | 113 (79.0)                    | 88 (61.5)                     | 26 (18.2)                         | exon 9 and 20 (HS) PCR-ARMS       |
| Jensen JD           | 2012                | Denmark   | HB (HER2+) | NR              | 118 (49.4)                    | NR                            | 61 (25.7)                         | exon 9 and 20 PA                   |
| Kalinsky K          | 2009                | USA       | HB     | NR              | 366 (62.0)                    | 314 (57.8)                    | 192 (32.5)                        | exon 1–20 SM + SS                  |
| Li SY               | 2006                | Australia | HB     | 59 (18–93)      | 168 (68.9)                    | 156 (63.9)                    | 88 (35.2)                         | exon 7, 9 and 20 F-SSCP          |
| Loi S               | 2013                | Finnish   | HB     | NR              | 475 (69.1)                    | NR                            | 174 (25.3)                        | exon 1, 2, 4, 9, 13, 18, 20 SM   |
| Pérez-Tenorio G     | 2007                | Sweden    | HB     | NR              | 188 (70.4)                    | NR                            | 65 (24.3)                         | exon 9 and 20 SSCP + DS           |
| Santarpia M         | 2008                | Italy/Spain | HB     | 58 (32–85)      | 44 (74.6)                     | 33 (55.9)                     | 17 (27.9)                         | exon 9 and 20 (HS) AD             |

NR, not reported; HB, hospital based group; HS, hotspots mutation; AD, allelic discrimination; DHPLC, denaturing high performance liquid chromatography; DS, direct sequencing; SNaPshot, SNaPshot genotyping assay; DxS, DxS PI3K mutation test kit; F-SSCP, fluorescent Single-Strand Conformation Polymorphism; PA, pyrosequencing assay; PCR-Amplification Refractory Mutation System (PCR-ARMS); SM, Sequenom MassARRAY; SS, Sanger sequencing.

* means that the ranges of age were not reported in the studies.
**Figure 2** | Forest plot with OR evaluating the relationship between **PIK3CA** mutation and ER expression status.

**Table 1**

| Study or Subgroup | ER positive | ER negative | Odds Ratio M-H. Fixed 95% CI |
|-------------------|-------------|-------------|-----------------------------|
| **1.1.1 DS Subgroup** |             |             |                             |
| Bachman KE, 2004  | 6           | 28          | 1.3% 0.82 [0.17, 4.00]      |
| Benvenuti S, 2008 | 19          | 65          | 27 1.0% 3.13 [0.86, 14.37]  |
| Bozhanov SS, 2010 | 24          | 80          | 21 6.3% 0.88 [0.43, 1.78]   |
| Czikova M, 2012   | 131         | 335         | 20 6.9% 3.11 [1.84, 5.29]   |
| Dunlop J, 2010    | 12          | 66          | 0 15 0.3% 7.11 [0.40, 126.87] |
| Li H, 2010        | 41          | 137         | 2 28 0.9% 5.55 [1.28, 24.98]|
| Liang X, 2006     | 17          | 37          | 13 40 2.6% 1.27 [0.70, 2.25]|
| Liedtke C, 2008   | 15          | 78          | 8 62 2.3% 1.61 [0.83, 4.08] |
| Lin CH, 2011      | 16          | 81          | 6 35 2.9% 1.19 [0.42, 3.55] |
| López-Knowles E, 2010 | 7       | 113         | 5 48 2.5% 0.57 [0.17, 1.89] |
| Mangone FR, 2012  | 18          | 53          | 5 27 1.7% 2.26 [0.73, 6.97] |
| Maryama N, 2007   | 42          | 124         | 12 64 4.0% 2.22 [1.07, 4.60]|
| Micheucci A, 2009 | 40          | 98          | 7 31 2.4% 2.36 [0.63, 0.91] |
| Saal LH, 2005     | 33          | 77          | 11 79 2.4% 4.04 [2.12, 10.12]|
| Sanchez C, 2011   | 14          | 32          | 1 17 0.3% 12.44 [1.47, 105.52]|
| Subtotal (95% CI) | 1434        | 696         | 37.9% 2.23 [1.76, 2.83]     |

Total events 435 116
Heterogeneity: $\chi^2 = 24.99$, df = 14 ($P = 0.03$); $I^2 = 44$
Test for overall effect: $Z = 8.80$ ($P < 0.00001$)

**Figure 3** | Forest plot with OR evaluating the relationship between **PIK3CA** mutation and PR expression status.

**Table 2**

| Study or Subgroup | PR positive | PR negative | Odds Ratio M-H. Fixed 95% CI |
|-------------------|-------------|-------------|-----------------------------|
| **1.2 Other sequencing methods** |             |             |                             |
| Barberaschi M, 2007 | 36         | 137         | 7 26 3.3% 1.04 [0.41, 2.68] |
| Butitta F, 2006    | 35          | 124         | 11 56 4.2% 1.61 [0.75, 3.48]|
| Campbell IG, 2004  | 15          | 32          | 7 19 1.8% 1.51 [0.47, 4.84] |
| Dupont Jensen J, 2011 | 37       | 78          | 6 19 1.9% 1.96 [0.87, 5.67] |
| Hartí A, 2013      | 20          | 93          | 6 24 2.0% 0.82 [0.29, 2.34] |
| Jensen JD, 2012    | 32          | 117         | 29 120 8.0% 1.18 [0.66, 2.12]|
| Kalinsky K, 2009   | 141         | 366         | 44 186 13.8% 2.02 [1.36, 3.01]|
| Li SY, 2016        | 68          | 168         | 18 76 5.6% 2.25 [1.22, 4.14] |
| Lori G, 2013       | 140         | 475         | 36 212 13.5% 2.04 [1.36, 3.08]|
| Pérez-Tenciio G, 2007 | 52     | 188         | 13 79 5.1% 1.89 [0.98, 3.81]|
| Santarpia M, 2008  | 12          | 44          | 5 15 2.1% 0.76 [0.21, 2.65] |
| Subtotal (95% CI)  | 1822        | 832         | 62.1% 1.74 [1.43, 2.11]     |

Total events 591 182
Heterogeneity: $\chi^2 = 8.55$, df = 10 ($P = 0.57$); $I^2 = 0$
Test for overall effect: $Z = 5.56$ ($P < 0.00001$)

**Figure 4** | Forest plot with OR evaluating the relationship between **PIK3CA** mutation and **HER2** expression status.

**Table 3**

| Study or Subgroup | HER2 positive | HER2 negative | Odds Ratio M-H. Fixed 95% CI |
|-------------------|---------------|---------------|-----------------------------|
| **1.3 Other sequencing methods** |             |               |                             |
| Barberaschi M, 2007 | 27          | 98           | 18 65 6.6% 0.99 [0.49, 2.00] |
| Butitta F, 2006    | 30           | 106          | 16 74 5.9% 1.43 [0.71, 2.87]|
| Hartí A, 2013      | 17           | 88           | 9 55 3.9% 1.22 [0.50, 2.96] |
| Kalinsky K, 2009   | 125          | 314          | 56 229 16.9% 2.04 [1.40, 2.98]|
| Li SY, 2006        | 63           | 156          | 24 88 7.9% 1.81 [1.02, 3.19]|
| Santarpia M, 2008  | 12           | 33           | 5 28 1.5% 2.40 [0.72, 8.00] |
| Subtotal (95% CI)  | 795          | 537          | 43.0% 1.69 [1.32, 2.16]     |

Total events 274 128
Heterogeneity: $\chi^2 = 4.29$, df = 5 ($P = 0.51$); $I^2 = 0$
Test for overall effect: $Z = 4.15$ ($P < 0.00001$)

**Figure 5** | Forest plot with OR evaluating the relationship between **PIK3CA** mutation and **HER2** expression status.
was no inter-study heterogeneity among the group B studies for RFS ($P = 0.93; F = 0\%$), a fixed-effect model was used to assess RFS correlations. For OS, 7 studies involving 2105 patients were analyzed and no significant association between PIK3CA mutations and OS was found (HR 1.14, 95% CI 0.72–1.82; $P = 0.57$) (Figure 4). We also performed analysis for different exons. For exon 9 mutations, a significant worse OS was found (HR 1.42, 95% CI 1.02–1.99; $P = 0.04$). In addition, for exon 20, the results of OS did not reach a significant level (HR 1.63, 95% CI 0.93–2.85; $P = 0.09$) (Figure 4). For RFS, 5 studies involving 1913 patients were analyzed, and a significant relationship between PIK3CA gene mutations and prolonged RFS was observed (hazard ratio 0.76, 95% CI 0.59–0.98; $P = 0.03$) (Fig. 5).

**PIK3CA gene mutations and prognosis in HR+ BCa patients.** The relationship between PIK3CA mutations and prognosis in HR+ BCa was evaluated in 8 studies involving 1021 patients, 5 studies (644 patients) for OS and 4 studies (534 patients) for RFS (group C) (Table 3). On the basis of the available data, kinase domain mutation is the priority for inclusion and analysis. No inter-study heterogeneity was found for OS ($P = 0.38; F = 4\%$) or RFS ($P = 0.73; F = 0\%$). PIK3CA gene mutations were not significantly associated with OS (hazard ratio 1.06, 95% CI 0.67–1.67; $P = 0.81$) (Fig. 6a) or RFS (hazard ratio 0.86, 95% CI 0.53–1.40; $P = 0.55$) (Fig. 6b) in HR+ BCa patients.

**Publication bias.** Publication bias was not investigated when the number of studies was less than 10 because of the low sensitivity of qualitative and quantitative tests. When the number of studies was more than 10, bias was assessed by Begg’s funnel plots. No evidence of obvious asymmetry was found in this analysis by visual evaluation (data not shown).

**Discussion**

Recently, several studies evaluating the prognosis of BCa patients suggest that PIK3CA mutations are “good mutations”. Our meta-analysis shows that PIK3CA gene mutations are significantly associated with both ER and PR expression, which are believed to be favorable clinicopathologic features of BCa. Furthermore, in unsorted BCa patients with PIK3CA mutations, RFS was significantly improved.

There are some possible explanations for the puzzling favorable effects of PIK3CA mutations. First, signaling pathways downstream of PI3K may not be active in some BCa patients with PIK3CA mutations. Loi et al. found that PIK3CA mutations were associated with relatively low mTORC1 signaling and that some AKT-regulated genes were repressed in BCa patients with PIK3CA mutations. Second, dysregulated gene expression resulting from PIK3CA mutations may be advantageous. Cizkova showed that the Wnt pathway was dysregulated and WNT5A was overexpressed in ER+ BCa patients with PIK3CA mutations. Interestingly, WNT5A expression has been associated with favorable outcomes in patients with invasive breast tumors. Third, PIK3CA, like many other oncopgenes, may induce senescence, resulting in a less aggressive phenotype after cell transformation.

Despite of this, there was only an insignificant connection between PIK3CA mutations and OS. The improvement in RFS but not OS may suggest a BCa specific effect of PIK3CA mutations. However, considering specific exons, the effects seemed weak or even contradictory. In the future, more studies focusing on specific exons mutations, including the non-hotspot mutations of PIK3CA, are warranted.

Whether PIK3CA mutations contribute to endocrine therapy resistance remains unclear and intriguing. Another important finding of this study was that PIK3CA mutations did not affect either OS or RFS in HR+ BCa patients. In most of the studies selected for our
analysis, hormone treatment was the standard therapy method. However, PIK3CA mutations may have only limited prognostic value with respect to hormone therapy responsiveness. Ellis et al. showed that the PIK3CA kinase domain mutations were inversely correlated with the clinical response to neoadjuvant endocrine treatment in BCa patients and was not associated with proliferation, as determined by immunostaining for Ki-67. In patients who did not receive tamoxifen, as Beelen et al. showed, PIK3CA mutation was not a prognostic marker, either.

It also should be noted that there is some dissociation between PIK3CA mutations and activation of signaling pathways downstream of PI3K. In some phase I clinical trials, PIK3CA mutations were not strongly related to responses produced by PI3K inhibitors. In our study, PIK3CA mutations were associated with favorable pro-

### Figure 4 | Forest plots of the analysis on the HR of OS in BCa patients. Subgroups are introduced for evaluating exon 9 or 20 mutations.

### Figure 5 | Forest plot of the analysis on the HR of RFS in BCa patients.
Table 3 | Main characteristics of studies that evaluated the relationships of PIK3CA mutations and the OS/RFS in HR+ breast cancer patients

| First author            | Year of publication | Country       | Design | Treatment | No. of PIK3CA mutant patients (%) | Sequenced PIK3CA mutations and the OS/RFS in HR+ breast cancer patients | Mutation analysis methods | Median follow-up time (months, range) | Outcome type |
|-------------------------|---------------------|---------------|--------|-----------|----------------------------------|------------------------------------------------------------------------|--------------------------|--------------------------------------|--------------|
| Bozhanov SS             | 2010                | Bulgaria      | HB     | H, C, RT  | 24 (100)                         | exon 9 and 20                                                          | DS                       | 69 (11-96)                           | OS           |
| Cuorvo LV               | 2014                | Italy         | HB     | H, C, T  | 50 (110)                          | exon 9, 10                                                             | HRM                      | 97 (8-140)                           | OS*          |
| PA                     | 97 (38)             | Italy         | HB     | H, C     | 50 (14)                           | exon 9, 10                                                             | MS                       | 118 (7-154)                          | OS           |
| Sanchez CG              | 2011                | USA           | HB     | H         | 13 (11)                           | exon 9, 10                                                             | DS                       | 51 (15-256)                          | OS*          |
| Ellis MJ                | 2010                | Multicentre   | HB     | H         | 45 (110)                          | exon 9, 20                                                            | DS                       | 45 (11-296)                          | OS*          |
| Maruyama N              | 2007                | Japan         | HB     | H         | 54 (28.7)                         | exon 9 and 20                                                          | DS                       | 64 (38-88)                           | OS           |

Note: *Only exon 20 mutations were analyzed.

In summary, our results show that PIK3CA mutations are significantly related to the ER and PR expression status of BCa patients. They also correlated with improved RFS in unsorted BCa patients, but not with OS or RFS in HR+ BCa patients. As a potential biomarker, PIK3CA mutations were not prognostic for HR+ BCa patients or, most notably, ER+ BCa patients. Further studies are needed to collectively explore the possible roles of PIK3CA mutations, the activation of signaling pathways downstream of PI3K, and other important biomarkers such as the genes encoding the components of the PI3K/AKT/mTOR pathway.

Methods

Literature search and eligibility criteria. We searched PubMed and Embase databases up to April 2014 for English-language titles or abstracts that included the words “phosphoinositide-3-kinase”, “PIK3CA”, “mutation”, “breast cancer”, or “breast neoplasms”. We also screened the references of the retrieved articles and relevant reviews for additional articles. A published article was included if it (1) evaluated the association between PIK3CA mutations and ER or PR expression in BCa patients or the association between PIK3CA mutations and BCa prognosis; (2) had sufficient data for estimating an OR with a 95% CI or a HR with a 95% CI; and (3) evaluated OS, RFS, or other survival index. The exclusion criteria were as follows: (1) letters, reviews, conference abstracts, and case reports; and (2) articles that did not provide sufficient information such as a HR for OS or had data that could not be extracted.

Data extraction and quality assessment. Two authors independently screened all publications by title or abstract for inclusion in our study. Discrepancies were resolved by group discussion, and data were extracted from eligible publications. The following information was collected: name of the first author, year of publication, source of patients, study design, mean age of the patients, percentage of ER+ and PR+ patients, percentage of patients with PIK3CA mutations, the region of the sequenced PIK3CA mutations, mutation analysis methods, outcome of BCa patients, and median follow-up time (months, range). The studies were assessed for quality according to the Newcastle-Ottawa quality assessment scale, and articles with 5 stars or more qualified for our study.

Statistical analysis. An OR with a 95% CI was used to assess the strength of the association between PIK3CA mutations and ER or PR expression status. The primary end points were RFS and OS. A HR and a 95% CI were used to estimate the impact of PIK3CA mutations on RFS and OS. When a HR and a 95% CI were not given in the article, estimated values were derived indirectly from Kaplan-Meier curves using the methods described by Tierney et al. Combined HR > 1 implied a worse survival for groups of patients with PIK3CA mutations. Cochran Q and I² statistic values were used to assess heterogeneity among the studies. For the Q statistic, a P value < 0.10 was considered statistically significant for heterogeneity, and the random-effects model was calculated according to the DerSimonian-Laird method. Otherwise, the fixed-effects model (Mantel-Haenszel method) was used. I² < 50% was considered acceptable. If significant heterogeneity was found, a random-effects model was used for meta-analysis. Statistical analyses were performed using
Review Manager 5.0 software (http://www.cochrane.org). A significant two-way $p$ value for comparison was defined as $P < 0.05$.

**Ethical Standards**

We declare that the experiments comply with the current laws of China.

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Author contributions
B.P. carried out the search of the Embase and Pubmed database, performed the statistical analysis by Revman, participated in the design of the study and drafted the manuscript. S.C. carried out the search of the Embase and Pubmed database and performed the statistical analysis by Revman. S.C. performed the data collection and extraction and helped to draft the manuscript. C.A. participated in the design of the study and made the language polishing. Z.Y.L. performed the data collection and arrangement. X.F. performed the data collection and arrangement. G.J.L. conceived of the study, and participated in its design and coordination and helped to draft the manuscript.

Additional information
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