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The prognostic value and potential drug target of phosphatase and tensin homolog in breast cancer patients

A meta-analysis

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Abstract

Background: The prognostic significance of phosphatase and tensin homolog (PTEN) in patients with breast cancer (BC) remains controversial. The aims of our meta-analysis are to evaluate its association with clinicopathological characteristics and prognostic value in patients with breast cancer.

Methods: PubMed, EMBASE, Web of Science, and China National Knowledge Infrastructure (CNKI) were systematically searched up to December 2016. The meta-analysis was performed using hazard ratio (HR), odds ratio (OR), and 95% confidence intervals (CI) as effect measures. A fixed or random effect model was used depending on the heterogeneity analysis. Statistical analysis was performed using Review manager software version 5.3.

Results: Seventeen studies including 4343 patients with breast cancer were analyzed. The meta-analysis indicated that breast cancers with PTEN loss were significantly associated with the tumor size ≥2 cm group (OR_{FEM} = 1.68, 95%CI_{FEM} [1.34, 2.10]), negative expression of estrogen receptor (OR_{REM} = 1.95, 95%CI_{REM} [1.09, 3.49]), negative expression of progesterone receptor (OR_{FEM} = 1.72, 95%CI_{FEM} [1.43, 2.08]), the advanced stage (OR_{REM} = 1.94, 95%CI_{REM} [1.35, 2.80]), positive axillary lymph node metastasis (OR_{REM} = 1.80, 95%CI_{REM} [1.30, 2.50]), and the local recurrence (OR_{FEM} = 1.70, 95%CI_{FEM} [1.26, 2.28]). None of other clinicopathological parameters such as the HER2 status and distant metastasis were associated with PTEN loss. The decreased PTEN expression was significantly correlated with the overall survival (OS) of patients (HR_{REM} = 1.83, 95%CI_{REM} [1.32, 2.53]) and the disease-free survival (DFS) of patients (HR_{REM} = 2.43, 95%CI_{REM} [1.31, 4.53]).

Conclusion: Our meta-analysis demonstrates that PTEN loss is of particular importance for predicting breast cancer aggressiveness and poor prognosis. PTEN is a potential drug target for the development of individualized treatment in BC patients.

Abbreviations: BC = breast cancer, CI = confidence interval, CNKI = China National Knowledge Infrastructure, CSC = cancer stem cell, DCIS = ductal carcinoma in situ, DFS = disease-free survival, ER = estrogen progesterone receptor, FEM = fixed effect model, HER-2 = human epidermal growth factor 2 receptor, HR = hazard ratio, IHC = immunohistochemistry, NA = not available, NOS = Newcastle–Ottawa Scale, OR = odds ratio, OS = overall survival, PI3K = phosphoinositide-3-kinase, PR = progesterone receptor, PTEN = phosphatase and tensin homolog, REM = random effect model, TMA = tissue microarray.

Keywords: breast cancer, meta-analysis, prognosis, PTEN

1. Introduction

Breast cancer (BC) is the most common malignancy and the leading cause of cancer death for females. Approximately 234,190 women in the United States are diagnosed with breast cancer, and 40,730 deaths occur in 2015.[1] Despite the development of surgery and adjuvant chemotherapy/endocrinotherapy have been improved over past few years, the long-term survival rates of BC patients are still poor. The prognostic factors that have been implicated include tumor size, estrogen/progesterone receptor (ER/PR), human epidermal growth factor 2 receptor (HER-2), axillary lymph nodes metastasis, tumor stage, local recurrence, and distant metastasis.[2,3] However, the molecular mechanism of the clinical outcome in BC patients is still not fully understood. Therefore, researchers are absorbed in identifying novel prognostic biomarkers and therapeutic targets for the management of breast cancer.

Much attention has been concentrated upon the loss of PTEN function in tumor development and progression. Phosphatase and tensin homolog (PTEN), located on chromosome 10q23.3, acts in the phosphoinositide-3-kinase(PI3K)/AKT/mTOR pathway as a

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tumor suppressor gene.[4] Previous studies have found that PTEN can block the activation of AKT through dephosphorylation of phosphatidylinositol-(3,4,5)-triphosphate (PIP3) generated by PI3K. Moreover, the PTEN/PI3K/AKT pathway is responsible for regulating the signaling of multiple biological processes such as cell proliferation, metabolism, apoptosis, and tumor angiogenesis.[5,6] Given these functions, aberrant loss of PTEN has been observed to be tightly linked to tumorigenesis and aggressive tumor behavior in cancer patients. The deletion of PTEN has been implicated in a number of human malignancies including glioma,[7] nonsmall cell lung cancer,[8] prostate tumors,[9] and breast cancer.[10] Several in vivo studies also demonstrated that deletion of PTEN gene led to massively increased susceptibility to multiple tumor types.[11]

Many retrospective studies have evaluated whether PTEN loss may be a potential predictor for outcome in patients with breast cancer. However, the studies measuring the alterations of PTEN gene in breast cancer specimens show highly discordant results.[12,13] In order to clarify the question, we collected and combined all eligible published articles to evaluate the value of PTEN as a prognostic biomarker for breast cancer and to determine the association between PTEN and several clinicopathological parameters of breast cancer.

2. Materials and methods

2.1. Search strategy and study selection

The electronic databases PubMed, EMBASE, Web of Science, and China National Knowledge Infrastructure (CNKI) were searched for studies to include in this meta-analysis up to December, 2016. The key words were searched as follows: "breast cancer" or "breast carcinoma" or "breast tumor" or "breast tumor" or "breast neoplasm" or "mammary gland cancer," "phosphatase and tensin homolog" or "PTEN," and "prognosis" or "survival" or "outcome." The study was approved by the Ethics Committee for human experiments of Capital Medical University.

To be eligible for inclusion in this meta-analysis, a study must meet the following criteria: (1) correlation between PTEN expression with BC patients' survival (i.e., overall survival [OS] and/or disease free survival [DFS]) was investigated; (2) correlation between PTEN and clinicopathological parameters of breast cancer was described; (3) the patients received either mastectomy or breast conservation treatment. None of them received neoadjuvant chemotherapy prior to surgery. Postoperative chemotherapy, radiotherapy, and hormone therapy were given according to the molecular subtype of breast cancer. The median follow-up period was no less than 24 months, and (4) all selected BC patients were pathologically confirmed.

The exclusion criteria were as follows: (1) nonhuman studies, (2) review articles, letters, or case reports, (3) duplicate/parallel publication, (4) with no more than 20 qualified BC patients, and (5) with insufficient data supply to calculate the hazard ratios(HR) and its 95% confidence interval (95%CI), or the Kaplan–Meier curve in the article could not be extracted. Titles and abstracts of all candidate manuscripts were carefully read by 2 independent authors (XF and JHC). Manuscripts that could not be categorized based on titles and abstracts were rechecked for the full-text review. To reach an agreement, disagreements on conflicting results were resolved between the 2 authors.

2.2. Data extraction

All relevant articles included were screened and assessed independently by 2 investigators (XF and JHC). To identify high-quality studies, each publication was scored based on the New-castle-Ottawa (NOS) Quality Assessment Scale.[14] Study with a score of 6 or higher was considered as a high quality study. Information was elaboratively extracted from the full publications, including the following items: first author, number of patients, year of publication, country of origin, detection method, cut-off value (positive PTEN expression), antibody for PTEN detection, analysis method (univariable or multivariable), hazard ratio (HR) for survival (OS and/or DFS), follow-up time, and quality assessment. If data from any of the above categories were not reported in the study, items were treated as "NA (not available)". To get the survival data that were not reported by the authors, we digitized and extracted the data from the Kaplan–-Meier curves in the articles using the software designed by Jayne F Tierney and Matthew R Sydes.[15]

2.3. Statistical methods

The enrolled studies were divided into 2 groups for analysis: those with data regarding OS and DFS. Hazard ratios (HRs) and 95% confidence intervals (CIs) were used to combine as the effective value. For the pooled analysis of the correlation between negative PTEN expression and clinicopathological features (tumor size, ER/PR/HER-2 status, axillary lymph node metastasis, tumor stage, local recurrence, and distant metastasis), odds ratios (ORs), and 95%CIs were combined to estimate the effect. I^2 and Q tests were performed to calculate the heterogeneity of the individual HRs/ORs. A probability value of $P < .1$ and $I^2 \geq 50\%$ indicated the existence of significant heterogeneity. If HRs/ORs were found to have fine homogeneity ($P > .1$ and $I^2 < 50\%$), a fixed effect model (FEM) was used for data analysis; if not, a random-effect model (REM) was used. An observed HR or OR >1 implied a worse prognosis in the negative PTEN expression group compared to positive PTEN expression group and would be considered to be statistically significant if the 95%CI did not overlap with 1. For these analyses, $P < .05$ was considered to indicate significance.

Publication bias was assessed using Begg's funnel plot and Egger's test. Sensitivity analyses were carried out to test the robustness of the results of meta-analysis. All the calculations were performed by Review Manager version 5.3 (Cochrane Collaboration, Oxford, England).

3. Results

3.1. Study selection and characteristics

A total of 337 potentially relevant manuscripts were reviewed, and a total of 17 studies[12,13,16–30] met the inclusion criteria using the search strategy mentioned above, comprising 4343 patients for final analysis (Fig. 1). The major clinical characteristics of the 17 eligible publications were reported in Table 1. The sample size of the included studies ranged from 34 to 1239 patients (median sample size, 255 patients). The studies were conducted in 11 countries (China, Denmark, Illinois, Saudi Arabia, Japan, Korea, Greece, Sweden, Germany, India, and the United States) and published between 2001 and 2016. Among the 17 studies, 13 studies were performed using the immunohistochemistry (IHC) method, and 1 study followed the tissue microarray (TMA) method. Eight studies reported the prognostic

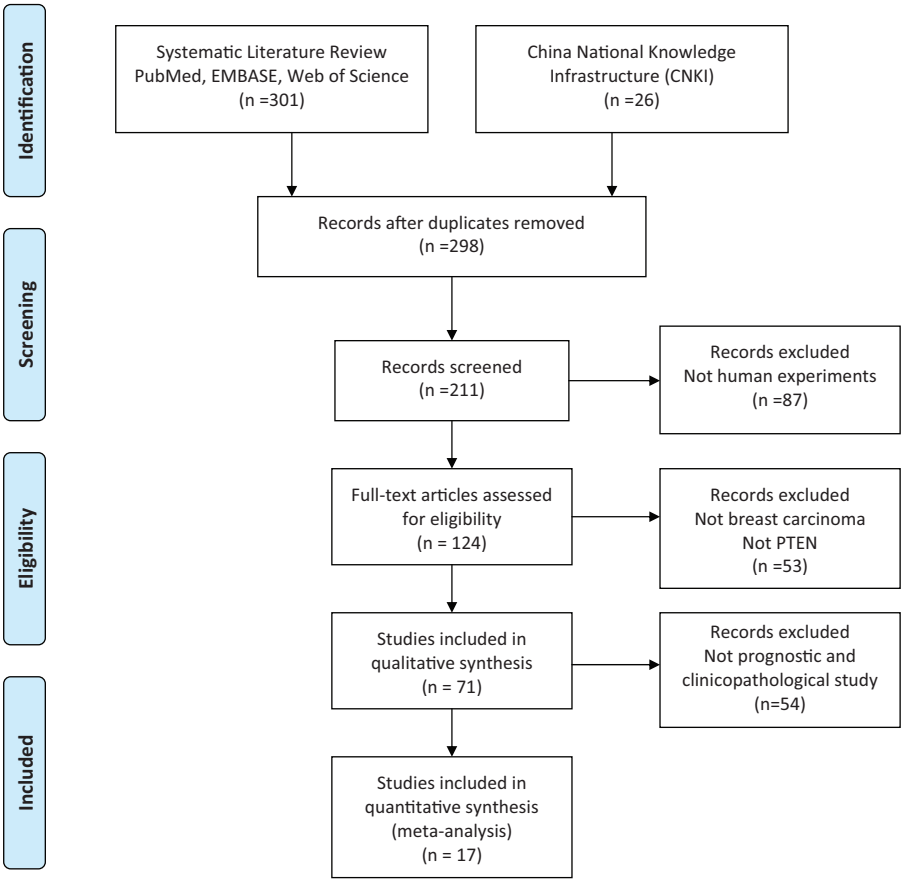

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Figure 1. PRISMA flow chart of the literature search.

value of PTEN status for survival in patients with breast cancer. Among them, 6 studies reported the overall survival of BC patients, and 4 for disease-free survival. The proportion of patients exhibiting PTEN loss in individual studies ranged from 18.8% to 77.0%. The cut-off values of IHC/TMA evaluation applied in the studies were not consistent. Hazard ratios with their 95%CIs were extracted from the graphical survival plots in 5 studies (univariable analysis) and reported directly in 3 studies (multivariate analysis). All patients in the eligible studies were determined by the pathological stage.

3.2. Association of PTEN loss with clinical parameters

Meta-analysis was performed on studies assessing the association between reduced PTEN expression and tumor size, ER status, PR status, HER2 status, axillary lymph node metastasis, tumor stage, local recurrence, and distant metastasis. The pooled ORs were 1.68 (95% CI_{FEM}: 1.34–2.10, $I^2=30\%$; $P_{FEM}=.20$), 1.95 (95% CI_{REM}: 1.09–3.49, $I^2=91\%$; $P_{REM}<.00001$), 1.72 (95% CI_{FEM}: 1.43–2.08, $I^2=43\%$; $P_{FEM}=.09$), 1.18 (95% CI_{REM}: 0.62–2.22, $I^2=86\%$; $P_{REM}<.00001$), 1.80 (95% CI_{REM}: 1.30–2.50, $I^2=71\%$; $P_{REM}<.0001$), 1.94 (95% CI_{REM}:1.35–2.80, $I^2=55\%$; $P_{REM}=.02$), 1.70 (95% CI_{FEM}: 1.26–2.28, $I^2=16\%$; $P_{FEM}=.31$), and 2.24 (95% CI_{FEM}:0.55–9.08, $I^2=76\%$; $P_{FEM}=.006$), respectively (Fig. 2). Decreased PTEN was found to be significantly associated with bigger tumor size, negative ER/PR status, positive axillary lymph node metastasis, the advanced stage, and local recurrence but not with HER2 status and distant metastasis. To explore the potential source of heterogeneity among studies, "metareg" command was conducted utilizing variables as year of publication, country, antibody catalog, and detection method. The results showed that no variable included in the meta regression contributed to the heterogeneity.

3.3. Association of PTEN loss with overall survival (OS) and disease-free survival (DFS)

Six studies assessed the association of PTEN gene with OS in human breast cancer. The pooled HR with the random effect model was 1.83 (95% CI_{REM}: 1.32–2.53; $I^2=63\%$; $P_{REM}=.02$) (Fig. 3), indicating low PTEN expression significantly predicts poor OS of patients with breast cancer. By the consistent immunohistochemical method, meta-analysis was performed on 4 studies assessing the association of PTEN immunoexpression with DFS in human breast cancer. The combined HR with the random effect model was 2.43 (95% CI_{REM}: 1.31–4.53; $I^2=75\%$; $P_{REM}=.007$). Similarly, PTEN deletion was also significantly associated with poor DFS in breast cancer. Due to limited studies, no subgroup analysis regarding OS and DFS was identified in the meta analysis.

Table 1

Main characteristics and results of the enrolled studies.

Authors (ref)	Year	Number of patients	Country	Detection method	Cut-off, positive PTEN expression	Antibody for PTEN detection	Analysis method	HR for survival, 95%CI	Follow up, mo	NOS scale
Bose et al [16]	2001	34	USA	IHC	NA (21)	NA	NA	NA	NA	5
Depowski et al [17]	2001	151	USA	IHC	≥15%(78)	A2B1	NA	NA	NA	5
Cho et al [18]	2003	105	Korea	IHC	NA (58)	NA	Univariable	Survival curve DFS:1.59 (1.02–2.48) OS:1.65 (1.06–2.56)	NA	7
Lin Q[19]	2003	61	China	IHC	>50% (32)	NA	Univariable	Survival curve OS:1.91 (1.00–3.65)	72 (60–84)	7
Chung et al[20]	2004	88	Korea	IHC	score>2 (60)	A2B1	NA	NA	NA	5
Lee et al[13]	2004	99	Korea	IHC	NA (72)	NA	Multivariable	Reported OS:5.91 (2.65–13.20) DFS:6.5 (3.11–13.58)	71 (51–105)	8
Bandyopadhyay et al[21]	2004	85	Illinois	IHC	>10% (63)	Rabbit polyclonal	Multivariable	Reported DFS:2.67 (1.15–6.20)	60	8
Tsutsui et al[22]	2005	236	Japan	IHC	NA (169)	NA	Multivariable	Reported DFS:1.54 (0.89–2.67)	80.4	8
Tokunaga et al[23]	2007	131	Japan	LOH analysis	<30% in the peak value in tumor DNA (100)	NA	NA	NA	NA	5
Perez-Tenorio et al[24]	2007	201	Sweden	IHC	NA (126)	Clone 17.A	NA	NA	NA	5
Yang et al[25]	2008	95	China	IHC	Score≥1 (63)	ZM 0221	Univariable	Survival curve OS:1.81 (1.09–3.01)	72	7
Bakarakos et al[26]	2010	215	Greece	IHC	NA (158)	28H6	NA	NA	203 (0–241)	6
Palimaru et al[27]	2013	175	Denmark	RT-qPCR	>22.98 (Median) (46)	NA	NA	NA	NA	5
Lebok et al[28]	2015	1239	Germany	FISH	Probe signals >10 (1006)	PTEN probe	Univariable	Survival curve OS:1.76 (1.31–2.37)	63 (1–176)	7
Li et al[29]	2015	291	China	IHC	Score≥1 (166)	6H2.1	NA	NA	NA	5
Beg et al[12]	2015	957	Saudi Arabia	TMA	H Score≥90 (220)	NA	Univariable	Survival curve OS:1.08 (0.69–1.69)	53 (30–77)	7
Siddiqui et al[30]	2016	180	India	IHC	>10% (98)	Clone 17.A	NA	NA	NA	5

CI = confidence interval, FISH = fluorescence in situ hybridization, HR = hazard ratio, IHC = immunohistochemistry, LOH = loss of heterozygosity, NA = not available, NOS = New-castle-Ottawa, PTEN = phosphatase and tensin homolog, RT-qPCR = real time-quantitative PCR, TMA = tissue microarray.

3.4. Publication bias and sensitivity analysis

No evidence of publication bias was found in the funnel plot as it seems to be symmetrical (Fig. 4). Sensitivity analysis was performed on the eligible studies. The enrolled studies were sequentially omitted to investigate whether any single study could have an influence on the pooled OS or DFS. The results showed that the stable overall HR was found to be not dominantly influenced by each individual study.

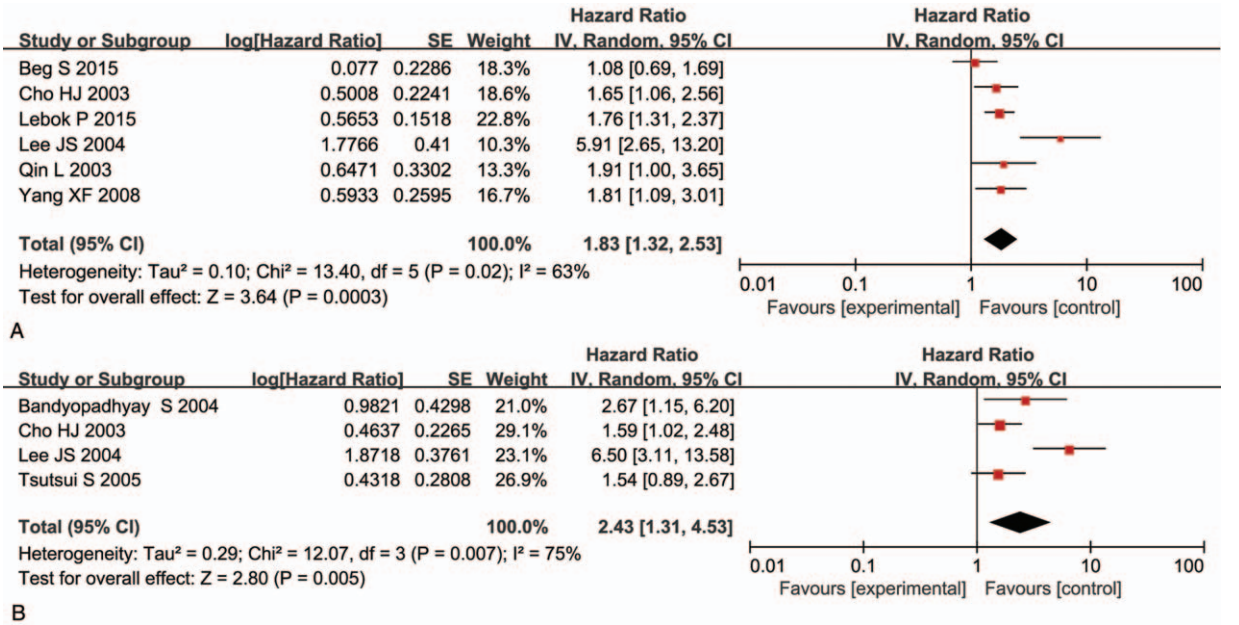

Figure 2. Forest plots of studies evaluating hazard ratios (HRs) of PTEN for overall survival (A) and disease-free survival (B) with the random effect model. HRs = hazard ratios, PTEN = phosphatase and tensin homolog.

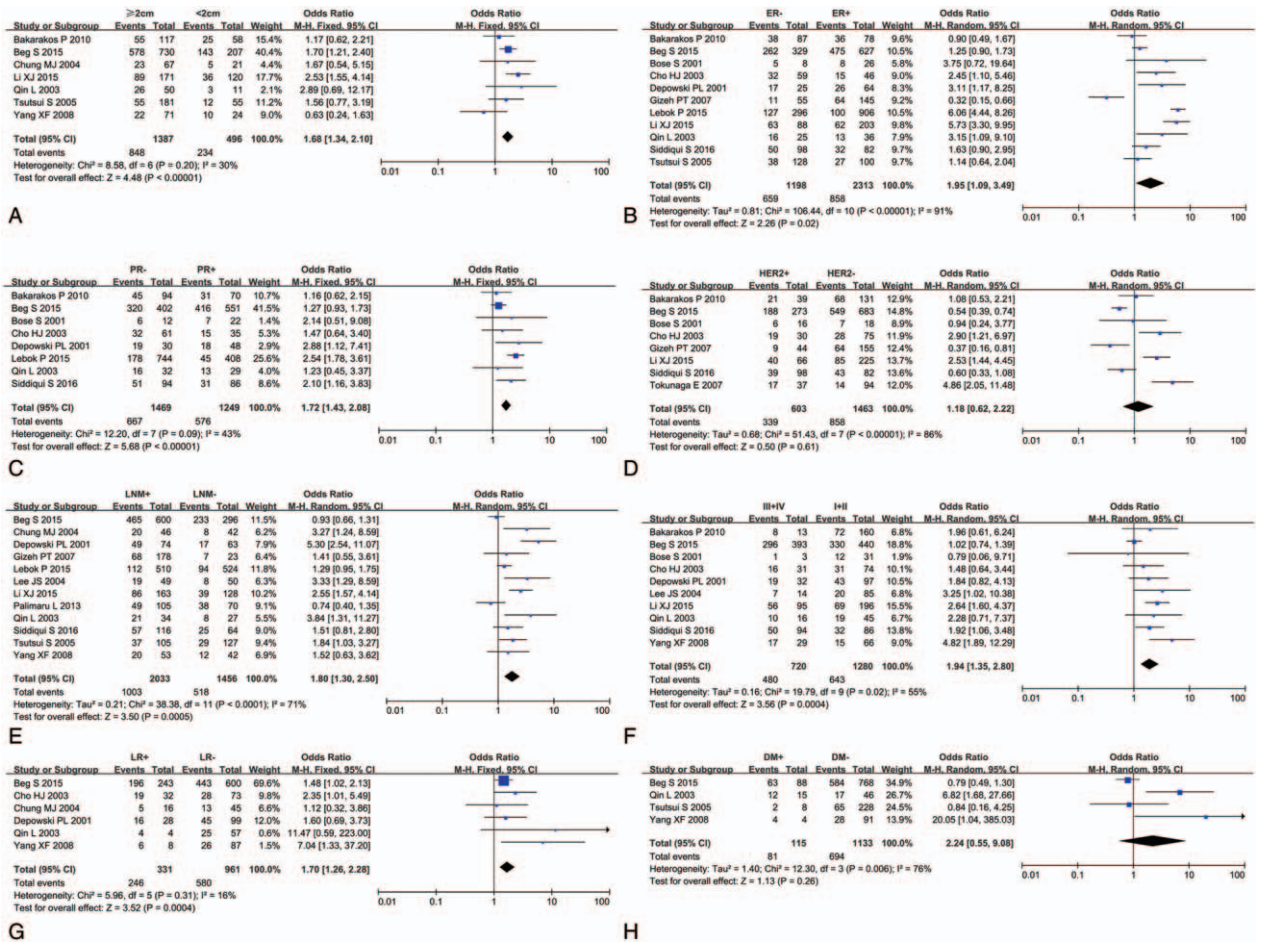

Figure 3. Forest plots of studies evaluating the association between PTEN and clinical parameters in breast cancer. (A) Tumor size (≥2 cm vs <2 cm), (B) ER status (negative vs positive), (C) PR status (negative vs positive), (D) HER-2 status (positive vs negative), (E) lymph node metastasis (present vs absent), (F) tumor stage (III +IV vs I+II), (G) local recurrence (present vs absent), (H) distant metastasis (present vs absent). ER = estrogen progesterone receptor, HER-2 = human epidermal growth factor 2 receptor, PR = progesterone receptor, PTEN = phosphatase and tensin homolog.

4. Discussion

Breast carcinoma is quite complex and heterogeneous in its carcinogenesis, progression, and response to treatment. In recent years, the frequency and relevance of PTEN alterations have been confirmed in primary and metastatic BC. Some studies on the treatment of this disease have revealed that PTEN loss is sufficient to cause a decreased response to trastuzumab in patients with HER2-overexpressing metastatic breast cancers.[31] Although the widely accepted role of PTEN in tumor development,[32] the prognostic value of PTEN in breast cancer is still controversial. As far as we know, our meta-analysis is the first to methodically elaborate the relationship between PTEN and clinicopathological characteristics and prognostic significance in breast cancer.

There were several meta-analyses studying the prognostic value of PTEN in other cancer types, such as lung cancer,[33] ovarian cancer,[34] glioma,[35] and prostate cancer.[36] In the present study, we combined 17 clinical studies indicating poor

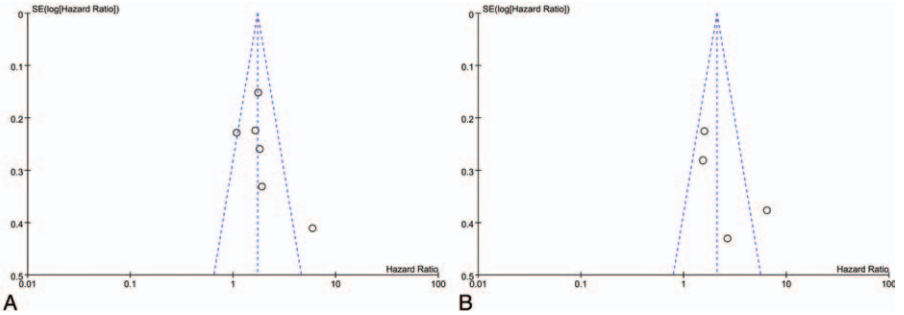

Figure 4. Funnel plots for all the included studies reported with OS (A) and DFS. (B). OS = overall survival, DFS = disease-free survival.

prognosis in BC patients with low expression of PTEN. The results showed that inactivated PTEN was significantly correlated with decreased 5-year OS and DFS rates of patients with mammary tumor. We applied meta-regression analysis to investigate the source of heterogeneity; however, none of the variables including detection method, antibody catalogue and analysis method contributed to the heterogeneity in our meta-analysis. Additionally, regarding the clinicopathologic features, decreased PTEN was found to be significantly associated with bigger tumor size, negative ER/PR status, positive axillary lymph node metastasis, the advanced stage, and local recurrence of breast carcinoma, symbolizing deterioration of the outcome. Based on these results, PTEN might act as a reliable biomarker in predicting clinical outcomes of breast carcinoma or as a potential drug target for the development of antitumor therapy on BC patients in future clinical trials.

The mechanisms responsible for the above association derived the following explanations. As a novel candidate tumor suppressor, PTEN plays an essential role in negative regulation of PI3K/AKT pathway,[37] which is involved in cell proliferation, invasion, and migration.[38,39] Recent studies indicated that PTEN loss might lead to normal stem cell exhaustion and the emergence and proliferation of cancer stem cell (CSC) clones.[40] Regarding PTEN loss, different genetic and epigenetic mechanisms including mutations, deletions, transcriptional silencing, or protein instability are involved in the regulation of PTEN inactivation. Among them, factors such as PTEN gene mutation and promoter methylation may be the dominant mechanism having a greater impact on PTEN-mediated tumorigenesis. It is reported that less than 4% PTEN intragenic mutations occurred in sporadic breast cancer.[41] Recent meta-analysis showed that PTEN promoter hypermethylation were considered to be one of the most important mechanism of PTEN inactivation in ductal carcinoma in situ (DCIS) and invasive ductal carcinoma of the breast, indicating PTEN inactivation was involved in an early stage of breast tumorigenesis.[42] Further study on the development of demethylation treatment is an area of research interest, which strongly supports that the PTEN gene is an important target for drug discovery.

Although our results are promising, our meta-analysis has several limitations. First, as a novel prognostic marker in breast cancer, the sample size of most studies was relatively small. Second, the high variability for PTEN protein expression reported by different authors could partly be attributable to no validated antibody, protocol of staining, and threshold used for PTEN immunoreactivity. Third, few studies explored the PTEN expression by some diagnostic methods other than IHC, which might bring out a certain publication bias. Fourth, some of the survival data were extracted from the Kaplan–Meier curves, which might introduce subjective bias. Fifth, the studies regarding OS and DFS were few in number. In the future, more multicentre studies with a larger sample size are required to present more reliable results of the clinical relevance for the abnormal expression of PTEN.

In conclusion, the present meta-analysis provided statistical evidence that PTEN downregulation can predict unfavorable breast cancer prognosis and aggressive tumor behavior. PTEN is a potential drug target for the development of individualized treatment in BC patients. Furthermore, well-designed prospective clinical studies should be performed to evaluate the application of PTEN for BC target therapy.

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