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ORIGINAL RESEARCH

The association of *PTEN* hypermethylation and breast cancer: a meta-analysis

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Objective: Phosphatase and tensin homolog (*PTEN*) deleted on chromosome 10, as a tumor suppressor gene, is crucial for the development of both familial and sporadic breast cancer (BC). The aim of this study was to perform a meta-analysis to evaluate the clinicopathological significance of *PTEN* promoter hypermethylation in BC.

Methods: A comprehensive literature search was made in PubMed, Embase, Google Scholar, Chinese database (China National Knowledge Infrastructure [CNKI]), and Web of Science. The analysis of pooled data was performed with Review Manager 5.2. The fixed-effects or random-effects models were used to evaluate odds ratios (ORs) and 95% confidence intervals (CIs).

Results: The meta-analysis included eight studies and a total of 923 patients. The frequency of *PTEN* promoter hypermethylation was significantly increased in ductal carcinoma in situ (DCIS) and invasive ductal carcinoma (IDC) compared to normal breast tissues (OR =22.53, P=0.0002 and OR =22.86, P<0.00001, respectively). However, the frequency of *PTEN* promoter hypermethylation was similar between IDC and DCIS. Additionally, *PTEN* methylation was not significantly correlated to estrogen receptor (ER) or human epidermal growth factor type 2 (HER-2) status in patients with BC.

Conclusion: *PTEN* promoter hypermethylation is significantly associated with the risk of DCIS and IDC, suggesting *PTEN* promoter hypermethylation is a valuable biomarker for diagnosis of BC.

Keywords: breast cancer, PTEN, meta-analysis, methylation, estrogen receptor, HER-2

Introduction

Breast cancer (BC) is the most common malignant disease in women worldwide, and the principal cause of cancer-related female mortality globally.[1] The development of BC involves a series of steps: initiating atypical ductal hyperplasia (ADH), followed by subsequent evolution to ductal carcinoma in situ (DCIS), culminating as invasive ductal carcinoma (IDC), and finally advancing to metastatic disease.[2] Molecular studies have demonstrated the great heterogeneity of IDC. Based on gene expression profiling and epithelial cell types, IDC is divided into four molecular subtypes: estrogen receptor (ER)-positive luminal A, ER-positive luminal B, ER-negative/human epidermal growth factor type 2 (HER-2), and basal subtype. Luminal A and B are most common subtypes, representing low- or intermediate-grade tumors. HER-2 and basal subtypes represent high-grade tumors and are characterized by lack of expression of ER and progesterone receptor (PR), displaying necrosis and lymphocytic infiltrate as well as poor response to treatment.[3] Given the variability in the clinical progression of disease, it becomes critical to identify markers for early detection of BC and prediction of tumor behavior.

Breast carcinogenesis is a stepwise accumulation of genetic changes including point mutations, deletions, oncogene activation, or tumor suppressor inactivation.[4]

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Epigenetics refers to heritable changes in gene expression that do not involve changes to the underlying DNA sequence and has received significantly more attention from researchers over the last decade.[5] Epigenetic alterations occur in malignant transformation which involves changes in DNA methylation, including global hypomethylation, focal hypermethylation, histone modifications, and nucleosomal remodeling.[6] Phosphatase and tensin homolog (*PTEN*) was deleted in breast cancer on chromosome 10, located on chromosomal subband 10q23.3, and is a tumor suppressor which is frequently mutated in human cancers.[7] PTEN regulates a variety of essential processes such as translation, cell cycle progression, and apoptosis by blocking the activation of the serine/threonine kinase Akt/PKB. Reduced or lost PTEN expression was observed in many human tumors, including brain tumors,[8] melanomas,[9] hepatocellular carcinomas,[10] thyroid carcinomas,[11] endometrial carcinomas,[12] lymphoid neoplasia,[13] and breast carcinomas.[14–17] Bose et al observed that the expression of PTEN protein is reduced or lost in 38% of invasive BCs.[14] Moreover, numerous studies have demonstrated that methylation is the mechanism for *PTEN* inactivation in BC.[18–21] The inactivation of *PTEN* has been considered to be caused by hypermethylation of its promoter in BC; the reported rates of *PTEN* promoter hypermethylation in BC are notably diverse. In addition, whether or not the inactivation is associated with the incidence and clinicopathological significance have not been thoroughly examined. The heterogeneous results of these studies do need further evaluation of the relationship between the promoter hypermethylation status of *PTEN* and BC. In this study, we quantified the association between *PTEN* promoter hypermethylation and BC using meta-analysis methods.

Methods

Selection criteria and study search

We comprehensively reviewed electronic databases including PubMed (1966 to January 2016), Web of Science (1945 to January 2016), Embase (1980 to January 2016), Cochrane Library Database (1972 to January 2016), Google Scholar, and Chinese database CNKI. The keywords "*PTEN* methylation" and "breast cancer" or "breast carcinoma" were used for relative studies searching without any language restriction. There were 48 studies identified from PubMed, 120 studies from Web of Science, 97 studies from Embase, and 350 studies from Google Scholar. A total of 615 studies were reviewed by titles and abstracts. We manually searched potentially relevant articles from the reference lists of retrieved articles.

Inclusion criteria were as follows: 1) studies about the relationship between *PTEN* methylation and the clinicopathological significance of BC and 2) studies about the association of *PTEN* methylation and prognosis in patients with BC. The following were the exclusion criteria: 1) studies that investigated the association between PTEN protein expression and clinicopathological significance, 2) articles that failed to show the diagnosis of histologic categories of BC, 3) studies that utilized the same population or overlapping database, and 4) studies that utilized cell lines or mice. Eight articles were eligible for inclusion in this meta-analysis. This meta-analysis was conducted in accordance with Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines.

Data extraction and study assessment

Two authors evaluated the included studies independently. Any disagreement was discussed and resolved by consensus. A standardized form was used to extract the data. The following information was collected from each study: first author's name, year of publication, geographical location, sample size of histologic categories of BC, ER status, stage of BC, HER-2 status, and detective method of *PTEN* methylation.

Statistics analysis

Odds ratios (ORs) with their 95% confidence intervals (CIs) were calculated. Heterogeneity among studies was evaluated using the Cochran's Q-statistic and I^2 tests. A fixed-effects model was used for $I^2<50\%$, while a random-effects model was used for $I^2>50\%$. Since all studies in the analysis were not equally precise, some studies were more precise than others we assigned more weight to the studies that contained more clinical information or data. In the fixed-effects analysis, each study was weighted by the inverse of its variance. In the random-effects analysis also, each study was weighted by the inverse of its variance. The difference is that the variance in random model included the original (within-studies) variance plus the between-studies variance, tau-squared. The analysis was conducted to compare the frequency of *PTEN* promoter hypermethylation between DCIS and normal tissue, IDC and normal tissue, and IDC and DCIS. In addition, we evaluated the correlation between *PTEN* promoter hypermethylation and ER, HER-2 status, and frequency of *PTEN* promoter hypermethyaltion in different stages. Two-sided statistical tests and P-value were used. Publication bias was evaluated using visual inspection funnel plots. All analysis was conducted with Review Manager 5.2.

Results

The meta-analysis included eight studies and a total of 923 patients (Figure 1). The basic characteristics are summarized in Table 1.

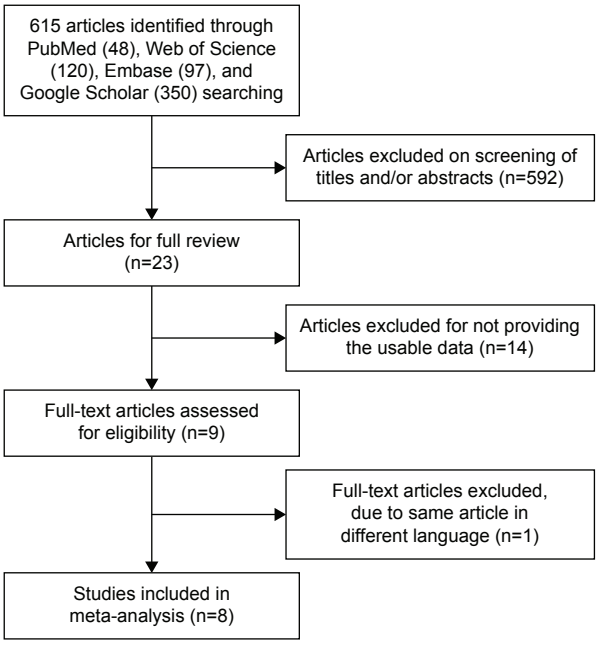

Figure 1 Schematic flow diagram for selection of included studies.

The frequency of *PTEN* promoter hypermethylation was significantly higher in DCIS than in normal breast tissues and the pooled OR was 22.53 (95% CI 4.35–116.72, z=3.71, P=0.0002, I^2=50%; Figure 2). *PTEN* promoter hypermethylation was significantly correlated to the risk of IDC (OR =22.86, 95% CI 11.09–47.09, z=8.48, $P<0.00001$, I^2=45%; Figure 3). The frequency of *PTEN* promoter hypermethylation was similar between IDC and DCIS (OR =1.39, 95% CI 0.50–3.87, z=0.63, P=0.53, I^2=64%; Figure 4). In addition, we found that the frequency of *PTEN* promoter hypermethylation was not significantly associated with stages of BC (OR =1.80, 95% CI 0.44–7.37, z=0.82, P=0.41, I^2=92%; Figure 5). The rate of *PTEN* promoter hypermethylation was similar between ER-positive and ER-negative status in patients with BC (OR =1.20, 95% CI 0.82–1.77, z=0.93, P=0.35, I^2=32%; Figure 6). No significant difference in the frequency of *PTEN* promoter hypermethylation was observed between HER-2-positive and HER-2-negative BC (OR =1.27, 95% CI 0.65–2.49, z=0.69, P=0.49, I^2=53%; Figure 7).

The Newcastle Ottawa Quality Assessment Scale (NOQAS) was used to assess the methodological quality of the included studies. This scale was used to allocate a total of nine points for the quality of selection (four points), comparability (two points), and exposure (three points). The NOQAS scores ranged from 0 to 9, and a score ≥7 indicates a good quality. Three studies scored eight points and five scored seven points (Table 2). A sensitivity analysis was performed by omitting a study at a time. The results were not significantly changed after removing a study at a time, indicating the stability of present analyses. The funnel charts were largely symmetric (Figure 8), suggesting no publication biases existed in the meta-analysis of *PTEN* promoter hypermethylation and clinicopathological features.

Discussion

BC is the most common form of malignant disease in women worldwide and is also the principal cause of cancer-related mortality globally. It is particularly important to identify molecular biomarkers for the early detection of the disease and development of appropriate treatment strategies.

PTEN is a dual lipid/protein phosphatase that regulates cell cycle progression, survival, cell growth, angiogenesis, and genomic stability through PI3K/Akt signaling pathway.[22–24] Its expression is modulated by germline and somatic *PTEN* mutation, genomic deletion, and promoter methylation silencing in many primary and metastatic tumors.[18,25–28] Germline mutations throughout the PTEN coding region were found in PTEN hamartoma tumor syndrome (PHTS), which is a rare disease, including the previously named Cowden syndrome and Bannayan-Rilev-Ruvalcaba syndrome.[29] PHTS patients have a high lifetime risk of developing breast, thyroid, or endometrial cancer due to *PTEN* silencing.[30] Lifetime risks of developing breast, ovary, colorectal, and

Table 1 Main characteristics of the included studies

Author	Year	Country	Sample (N)			Stage (I+II/III)	ER Status (–/+)	HER-2 status (–/+)	Methods
			Normal	DCIS	IDC				
Garcia et al[20]	2001	Spain	90	18	72	61/29	38/52	22/21	MSP
Khan et al[21]	2004	USA	16		50				MSP
Klajic et al[48]	2013	Norway	6	21	101	85/103			PS
Muggerud et al[49]	2010	Norway	28	27	28				PS
Zhang et al[54]	2013	People's Republic of China	10		45	28/17	25/20	33/12	MSP
Zhao et al[58]	2010	People's Republic of China	16		92				MSP
Yari et al[59]	2016	Iran	50		103		27/70	23/34	MSP
Siddiqui et al[60]	2016	India	180		180	39/32	33/38	32/39	MSP

Abbreviations: MSP, methylation-specific PCR; PS, pyrosequencing; PCR, polymerase chain reaction; DCIS, ductal carcinoma in situ; IDC, invasive ductal carcinoma; ER, estrogen receptor; HER-2, human epidermal growth factor type 2.

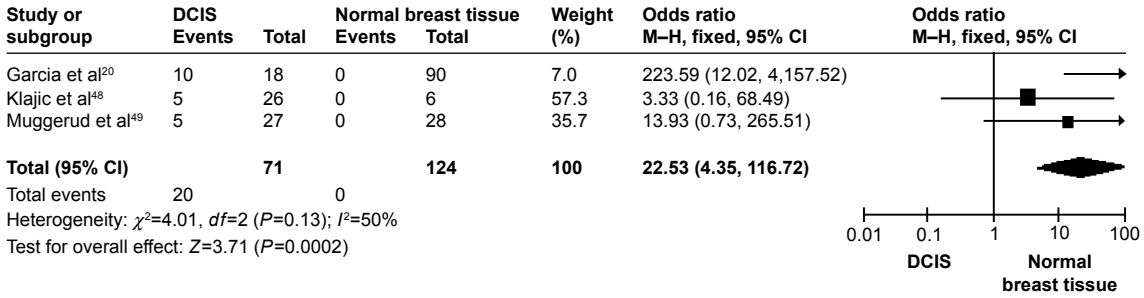

Figure 2 Forest plot for *PTEN* promoter hypermethylation in DCIS and normal breast tissue.
Abbreviations: DCIS, ductal carcinoma in situ; CI, confidence interval; M–H, Mantel–Haenszel.

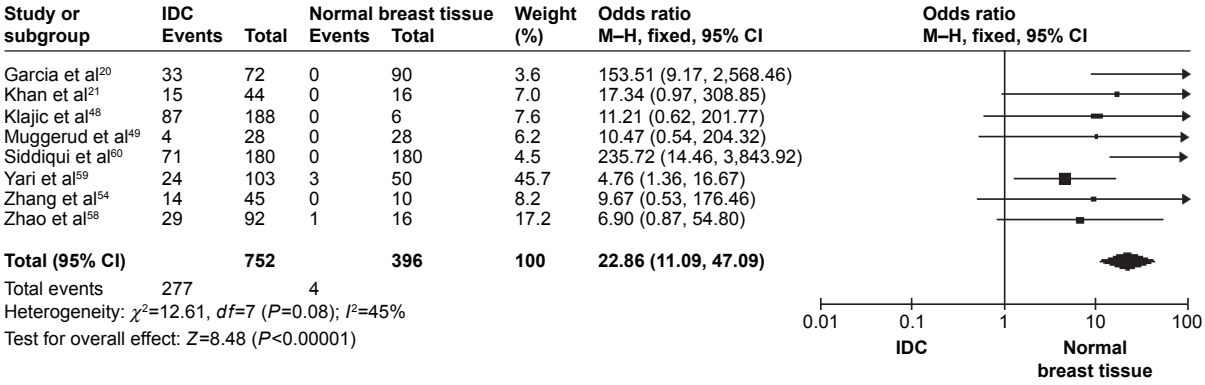

Figure 3 Forest plot for *PTEN* promoter hypermethylation in IDC and normal breast tissue.
Abbreviations: IDC, invasive ductal carcinoma; CI, confidence interval; M–H, Mantel–Haenszel.

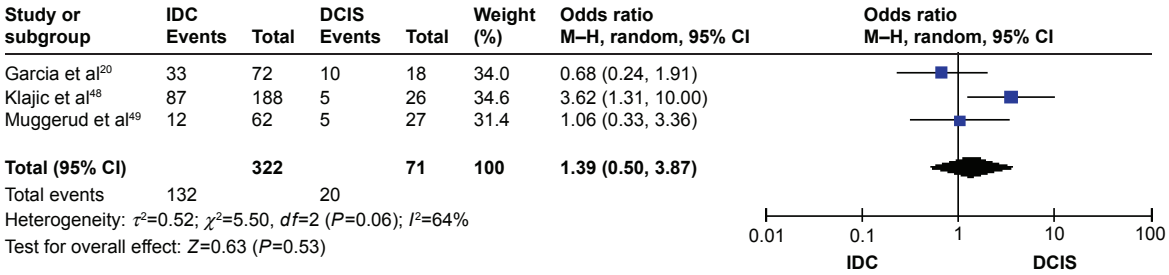

Figure 4 Forest plot for *PTEN* promoter hypermethylation in IDC and DCIS.
Abbreviations: DCIS, ductal carcinoma in situ; IDC, invasive ductal carcinoma; CI, confidence interval; M–H, Mantel–Haenszel.

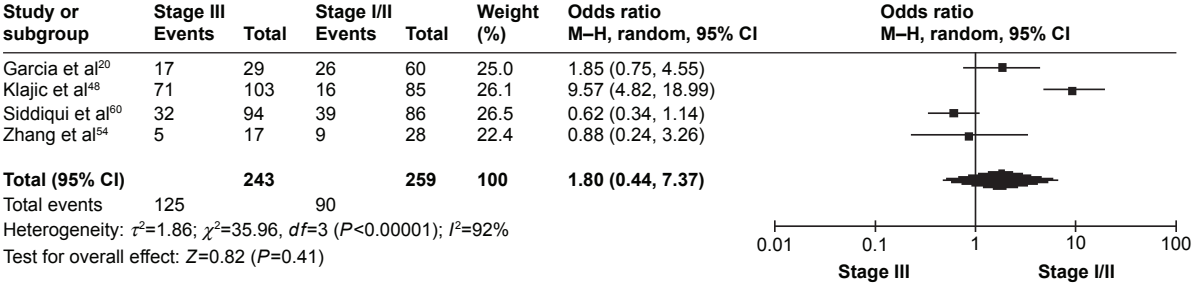

Figure 5 Forest plot for *PTEN* promoter hypermethylation in different stages of BC.
Abbreviations: BC, breast cancer; CI, confidence interval; M–H, Mantel–Haenszel.

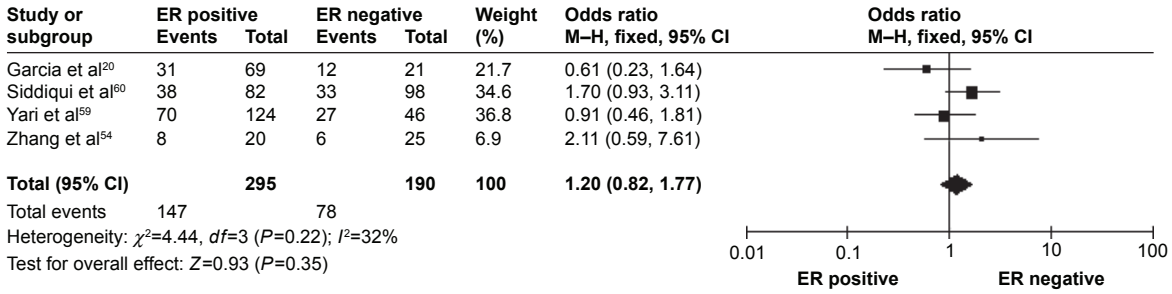

Figure 6 Forest plot for *PTEN* promoter hypermethylation in ER-positive and ER-negative BC.
Abbreviations: BC, breast cancer; CI, confidence interval; ER, estrogen receptor; M–H, Mantel–Haenszel.

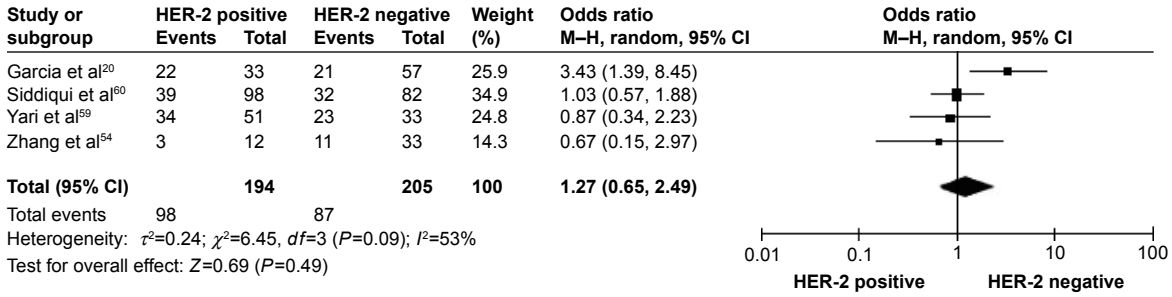

Figure 7 Forest plot for *PTEN* promoter hypermethylation in HER-2-positive and HER-2-negative BC.
Abbreviations: BC, breast cancer; CI, confidence interval; HER-2, human epidermal growth factor type 2; M–H, Mantel–Haenszel.

Table 2 Quality assessment according to the Newcastle Ottawa Quality Assessment Scale of the included studies

Author	Selection	Comparability	Exposure	Total score
Garcia et al[20]	2	2	3	7
Khan et al[21]	2	2	3	7
Klajic et al[48]	3	2	3	8
Muggerud et al[49]	3	2	3	8
Zhang et al[54]	2	2	3	7
Zhao et al[58]	2	2	3	7
Yari et al[59]	3	2	3	8
Siddiqui et al[60]	2	2	3	7

Note: This scale was used to allocate a total of nine points for the quality of selection (four points), comparability (two points), and exposure (three points).

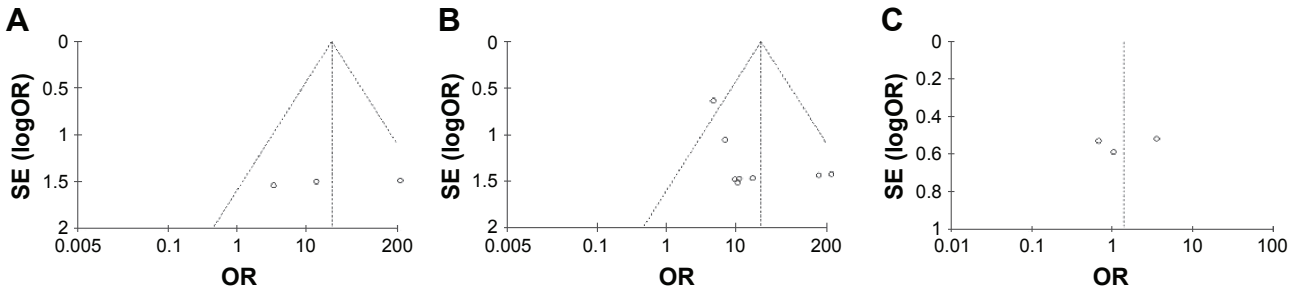

Figure 8 Funnel plot for publication bias.
Notes: Each circle represents a separate study for the indicated association. Log(OR) = natural logarithm of OR. (**A**) *PTEN* promoter hypermethylation in DCIS and normal breast tissue; (**B**) *PTEN* promoter hypermethylation in IDC and normal breast tissue; (**C**) *PTEN* promoter hypermethylation in IDC and DCIS.
Abbreviations: OR, odds ratio; SE, standard error; DCIS, ductal carcinoma in situ; IDC, invasive ductal carcinoma.

kidney cancers and melanoma increase in patients with germline *PTEN* mutations.[31] *PTEN* promoter hypermethylation was reported in cervical cancer,[32] colorectal cancer,[33,34] esophageal squamous cell carcinoma,[35] gastric cancer,[36,37] hepatocellular carcinomas,[38] head and neck cancer,[39] lung cancer,[40–42] and ovarian cancer.[43] A frequency of less than 4% *PTEN* intragenic mutations were observed in sporadic BC.[44–47] Therefore, *PTEN* promoter hypermethylation has been considered to be one of the most important mechanisms of inactivation. Several studies have reported more frequent *PTEN* promoter hypermethylation in DCIS than in normal breast tissue with inconsistent rate.[20,48,49] We pooled those studies together and more precisely evaluated the frequency of *PTEN* promoter hypermethylation in DCIS and normal breast tissue. Our results showed *PTEN* promoter hypermethylation was significantly correlated with the risk of DCIS, suggesting *PTEN* promoter hypermethylation is an early event during breast carcinogenesis. Barekati et al investigated the promoter methylation of *PTEN* in triple-matched samples from BC, including cancerous tissue, matched adjacent normal tissue and serum samples, and found that the levels of *PTEN* methylation in tumor tissue and serum of patients was significantly increased compared to those in the normal breast tissue and serum, respectively.[50] These data indicate that *PTEN* hypermethylation is a valuable biomarker for diagnosis of BC.

Interestingly, *PTEN* loss is more frequently observed in triple-negative BC than other subtypes of BC.[51,52] Lately, Beg et al have reported that loss of PTEN expression correlated with invasive behavior and poor prognosis in Middle Eastern triple-negative BC, a type of high-grade BC with poor response to treatment.[53] Thus, PTEN could be a novel target for the development of effective therapy, especially for patients with triple-negative BC. Further investigations need to be carried out in future. Additionally, the frequency of *PTEN* promoter hypermethylation in IDC was significantly increased compared to normal breast tissue. However, there was no significant difference between DIC and DCIS, and moreover *PTEN* was not frequently hypermethylated in early stage than late stage of BC patients, suggesting *PTEN* does not exhibit stage-dependent methylation pattern. Additional studies with a large number of patients are needed to clarify the relationship between *PTEN* promoter hypermethylation rate and tumor stages in the future.

In this study, we evaluated *PTEN* promoter hypermethylation in ER-positive versus ER-negative BC and showed similar frequency of hypermethylation, which is in line with previous studies.[20,54] Additionally, HER-2, a transmembrane tyrosine kinase receptor, belongs to a family of epidermal growth factor receptors structurally related to epidermal growth factor receptor.[55] HER-2 overexpression is observed in 20%–30% of BC cases and has been recognized as a poor marker of prognosis.[56,57] Klajic et al found significantly higher *z*-scores of *PTEN* promoter hypermethylation in HER-2-positive than in HER-2-negative BCs.[48] In the present study, there was no significant difference in the rate of *PTEN* promoter hypermethylation between HER-2-positive and HER-2-negative BCs. Due to the limited power of present study, more investigations need to be performed in the future. The present study included only the articles published in English or Chinese, and excluded relevant studies published in other languages. Therefore, our results should be interpreted with caution when applied to the general populations.

In summary, *PTEN* promoter is more frequently hypermethylated in DCIS and IDC compared to normal breast tissue, suggesting that PTEN is a valuable biomarker for diagnosis of BC. The frequency of *PTEN* promoter hypermethylation is similar between IDC and DCIS, indicating *PTEN* does not exhibit stage-dependent methylation pattern. In addition, it is likely that *PTEN* promoter hypermethylation is not significantly correlated with ER or HER-2 status in patients with BC.

Author contributions

All authors contributed toward data analysis, drafting and critically revising the paper and agree to be accountable for all aspects of the work.

Disclosure

The authors report no conflicts of interest in this work.

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