Int J Clin Exp Med 2015;8(1):616-624
www.ijcem.com /ISSN:1940-5901/IJCEM0003388

Original Article

Clinicopathological and prognostic role of SIRT1 in breast cancer patients: a meta-analysis

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Received October 25, 2014, Accepted January 7, 2015; Epub January 15, 2015; Published January 30, 2015

Abstract: Background: Silent mating type information regulation 2 homolog-1 (SIRT1) plays an important role in the progression and development of cancer, including breast cancer. However, the association between SIRT1 expression and clinicopathological parameters and prognosis in breast cancer remains inconclusive. To accurately evaluate the significance of SIRT1 expression in breast cancer, a meta-analysis based on published studies was performed. Methods: The PubMed, Embase, ISI Web of Science, Science Direct, and Chinese National Knowledge Infrastructure databases were screened to retrieve relevant literature. The reported odds ratios (ORs) and hazard ratios (HRs) and their 95% corresponding confidence intervals (CIs) were pooled to estimate the strength of specific associations. Results: Six studies involving 604 patients were included in the meta-analysis. The pooled analyses showed a significant correlation between SIRT1 expression and poor disease-free survival (DFS) (HR = 3.07, 95% CI: 1.92-4.91, Z = 4.69, P < 0.001) and overall survival (OS) (HR = 3.94, 95% CI: 2.19-7.10, Z = 4.57, P < 0.001). SIRT1 expression also significantly correlated with high TNM stage (pooled OR = 2.92, 95% CI: 1.84-4.63) and lymph node metastasis (pooled OR = 3.22, 95% CI: 0.98-10.57). Conclusions: Our meta-analysis shows that SIRT1 expression correlates with unfavorable clinical outcomes. We suggest that SIRT1 expression may have potential value in the pathological diagnosis and clinical treatment of patients with breast cancer. More studies are warranted to investigate the effect of SIRT1 on the survival of breast cancer patients.

Keywords: SIRT1, breast cancer, prognosis, meta-analysis

Introduction

Despite rapid development of new treatments in recent years, breast cancer remains the leading cause of cancer-related death among women worldwide [1, 2]. Aberrant epigenetic regulation of gene expression underlies cancer formation and progression [3], and numerous studies of genomic deacetylation link histone deacetylases to clinicopathological outcomes and prognosis in breast cancer [4].

Silent mating type information regulation 2 homolog-1 (SIRT1), a member of the sirtuin family (SIRT1-7), is a nicotinamide adenine nucleotide-dependent histone deacetylase [5-7]. SIRT1 extensively participates in a variety of physiological processes, such as gene silencing, metabolism, and genomic stability. Recent studies show that it plays a pivotal role in tumorigenesis and the proliferation, survival, and death of cancer cells [3, 8, 9]. SIRT1 expression is frequently elevated in many cancers, including prostate cancer [10], gastric cancer [11], and ovarian epithelial tumors [12], and SIRT1 is considered a tumor promoter. However, reduced expression of SIRT1 has been found in hepatic carcinomas and breast cancers [13]. The role of SIRT1 in cancer is therefore complex and requires further determination.

Considerable attention has been focused on SIRT1 expression in breast cancer in the past few years. However, the clinicopathological and prognostic significance of SIRT1 expression in breast cancer remains incompletely defined, and inconsistent results have been obtained. Previous studies [14, 15] reported that SIRT1 expression significantly correlated with lymph node status and markedly shortened disease-free survival (DFS) and overall survival (OS) times in breast cancer patients. Other studies

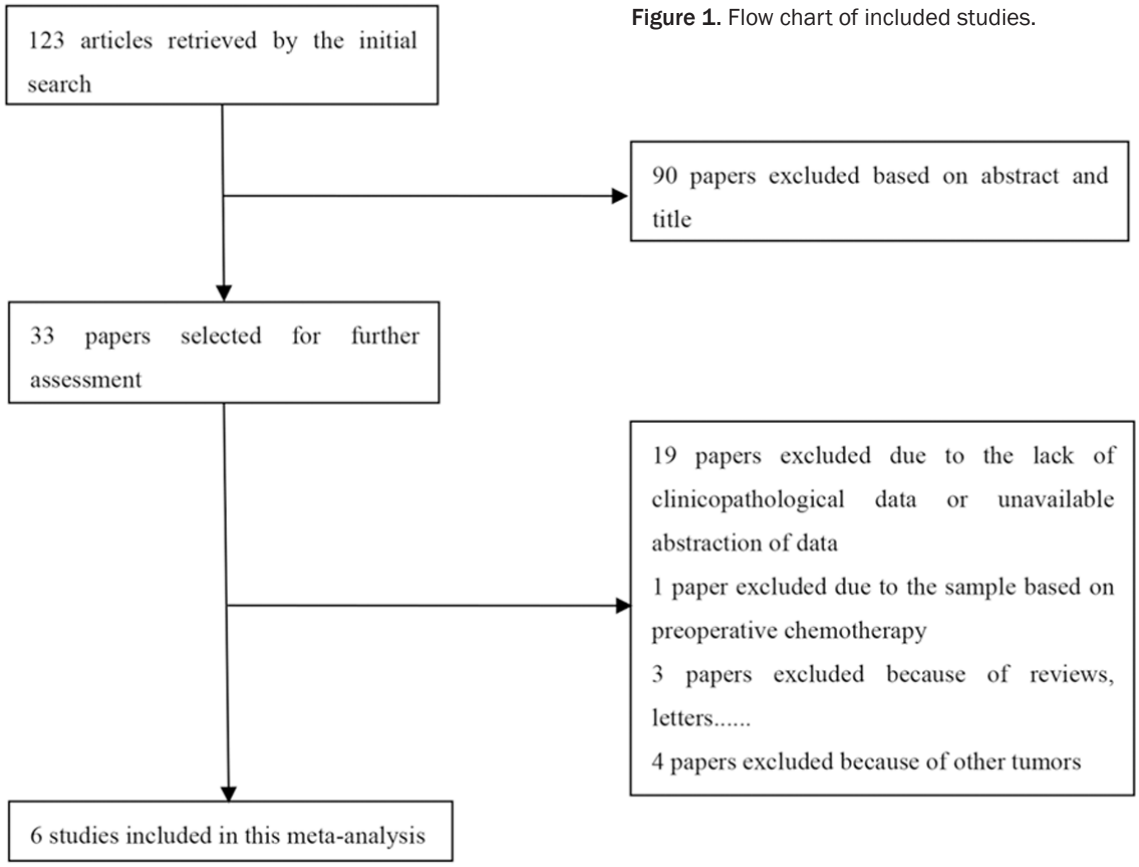

Figure 1. Flow chart of included studies.

[9, 16], however, showed no correlation of SIRT1 expression with lymph node metastasis and poor survival. These discrepancies may reflect the limited number of cases in previous studies. Hence, we thought it necessary to perform a meta-analysis to systematically qualify the clinicopathological and prognostic significance of SIRT1 expression in patients with breast cancer.

Materials and methods

Search strategy and study selection

We conducted an electronic search for relevant studies in the PubMed, Embase, Web of Science, and Chinese National Knowledge Infrastructure databases (up to July, 2014) without restriction of origin or language, using various combinations of the following terms: (1) "SIRT1" or "silent mating type information regulation 2 homolog-1" AND (2) "breast cancer" and "breast carcinoma" AND (3) "prognosis" or "prognostic" or "survival". For inclusion in our meta-analysis, a study was required to meet the following criteria: (1) SIRT1 expression was measured via immunohistochemistry in breast cancer tissue; (2) information on the association of SIRT1 expression with clinicopathological characteristics or prognosis was provided; and (3) the diagnosis of breast cancer was histologically and pathologically confirmed. We also manually searched the reference lists of the included studies to further retrieve potentially relevant studies. If 2 or more studies examined overlapping populations, only the most recently published study or the one with largest sample size study was selected.

Data extraction and literature quality assessment

To decrease bias and improve reliability, all data were extracted by 2 independent reviewers using a standardized data abstraction tool. The data extracted were as follows: name of the first author, year of publication, country of origin, histological type of breast cancer, number of participants, the choice of cutoff scores for the definition of positive staining or staining

Table 1. Characteristics of eligible studies

First author	Year	Country	Histology type	Number of patients	Cut-off value score	Clinicopathological factors	5-year DFS	5-year OS	NOS score
							HR (95% CI)	HR (95% CI)	
Sung [9]	2010	Korea	Mixed	28	CS	LNM, TNM, Grade, ER, PR, HER-2	NA	NA	6
Wu [14]	2012	China	Mixed	134	CS	Tumor size, LNM TNM, Grade	3.352 (1.586-7.087)	4.379 (1.657-11.568)	8
Song [15]	2013	China	Mixed	90	> 5%	Tumor size, LNM, TNM, Grade, ER, PR, HER-2	3.739 (1.283-10.893)	3.929 (1.387-11.129)*	7
Lee [16]	2010	Korea	Mixed	122	> 30%	Tumor size, LNM, TNM, Grade, ER, PR, HER-2	2.586 (1.250-5.349)	3.491 (1.221-9.979)	8
Zhu [20]	2011	China	IDC	30	> 30%	Tumor size, LNM, TNM, ER, PR, HER-2	NA	NA	5
Wan [21]	2014	China	IDC	200	CS	LNM, TNM, Grade, ER, PR, HER-2	NA	NA	6

IDC, invasive ductal carcinoma; Cut-off value, the boardline value to define expression of SIRT1; CS, complex score combining intensity and percentage; LNM, lymph node metastasis; NA, not available; *the HR and 95% CI were obtained according to methods previously described by Parmar [22].

Table 2. Meta-analysis of the associations between SIRT1 expression and clinicopathological characteristics in breast cancer

Clinical feature	Number of patients	Pooled OR (95% CI)	*P*-value	I^2 (%)	P_h
Tumor size	282	1.79 (0.63-5.12)	0.28	61%	0.050
LNM	604	3.22 (0.98-10.57)	0.05	88%	< 0.001
TNM stage	470	2.92 (1.84-4.63)	< 0.01	14%	0.320
Grade	604	1.74 (0.78-3.89)	0.44	66%	0.020
ER	514	1.19 (0.82-1.75)	0.36	43%	0.130
PR	504	1.19 (0.41-3.49)	0.75	79%	< 0.001
HER-2	524	1.30 (0.83-2.04)	0.26	24%	0.260

LNM, lymph node metastasis; P_h, *P*-value of heterogeneity.

intensity, clinicopathological parameters [including tumor size, lymph node metastasis (LNM), TNM stage, histological grade, and estrogen receptor (ER), progesterone receptor (PR), and HER-2 status], and DFS and OS.

Two reviewers separately assessed the quality of each study using the Newcastle-Ottawa Scale criteria described in previous studies [17, 18]; when a discrepancy arose, a third reviewer was consulted to resolve the dispute. Briefly, these criteria were as follows: (1) subject selection, scored 0-4; (2) comparability of subject, scored 0-2; and (3) clinical outcome, scored 0-3. Scores ranged from 0 (lowest) to 9 (highest), and studies with scores of 6 or more were rated as high quality [18].

Statistical analyses

Odds ratios (ORs) and their 95% confidence intervals (CIs) were used to assess correlations between SIRT1 expression and the clinicopathological features of breast cancer, including tumor size, LNM, TNM stage, histological grade, and ER, PR, and HER-2 status. Hazard ratios (HRs) and their 95% CIs were used for analysis of the effects of SIRT1 expression on OS and DFS; in these analyses, $P < 0.05$ was considered significant. All analyses were performed using RevMan 5.2 software (provided by The Cochrane Collaboration, Oxford, England). Heterogeneity among studies was evaluated using the chi-squared-based Q-test and the I^2 test. If there was no significant heterogeneity among studies (indicated by $P > 0.1$ in the Q test and an $I^2 < 50\%$), the pooled ORs and HRs of each study were calculated by the fixed-effects model (Mantel-Haenszel method). If heterogeneity was indicated, the random-effects model (the DerSimonian and Laird method) was adopted [19].

Evidence of publication bias was determined using Begg's test and Egger's test and Stata 12.0 software (Stata Corporation, College Station, TX, USA), and $P < 0.05$ was considered representative of statistically significant publication bias. All *P* values were 2-tailed.

Results

Search results

Initially, 123 articles were identified by searches of the PubMed, Embase, ISI Web of Science, Embase, and Chinese National Knowledge Infrastructure databases using the strategy described above. After reviewing the titles and abstracts, 90 studies were excluded because of duplication, irrelevancy, or non-originality (e.g., reviews, letters). The remaining 33 studies were reviewed in detail. Ultimately, 6 studies involving 604 patients with breast cancer were included in our pooled analyses [9, 14-16, 20, 21]. A flow chart of the included studies is shown in **Figure 1**.

Characteristics of the eligible studies

The characteristics of the 6 eligible studies are listed in **Table 1**. The publication years of the eligible studies ranged from 2010 to 2014. Four studies were conducted in China and 2 in Korea. The number of patients in each study ranged from 28 to 200 (mean sample size, 101 patients). All 6 studies were assessed for quality according to the Newcastle-Ottawa Scale. The quality of the studies varied from 5 to 8, with a mean of 7, and 5 studies had scores of 6 or more, indicating that they were of high quality. **Table 1** also lists the clinicopathological features of the patients in the 6 studies, including tumor size, LNM, TNM stage, grade, and ER, PR, and HER-2 status.

Meta-analysis

In all 6 studies, SIRT1 expression was independently evaluated by 2 or more pathologists who were unaware of the clinical data. In 3 of the 6

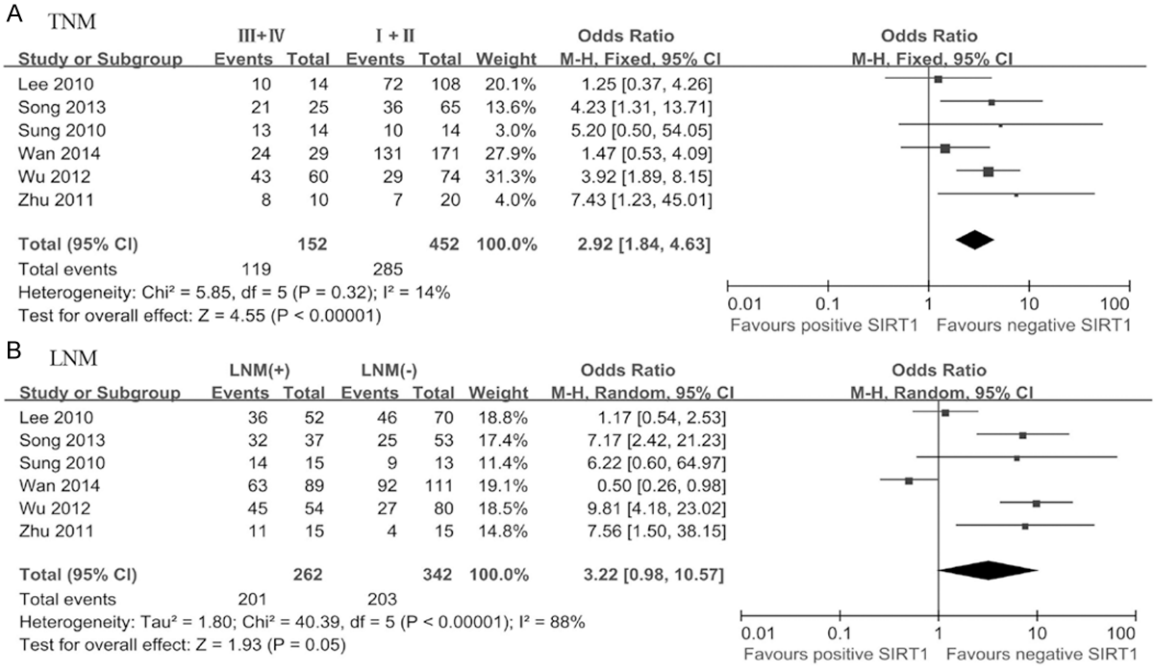

Figure 2. Forest plots of associations between SIRT1 expression with TNM (A) and LNM (B) in breast cancer patients.

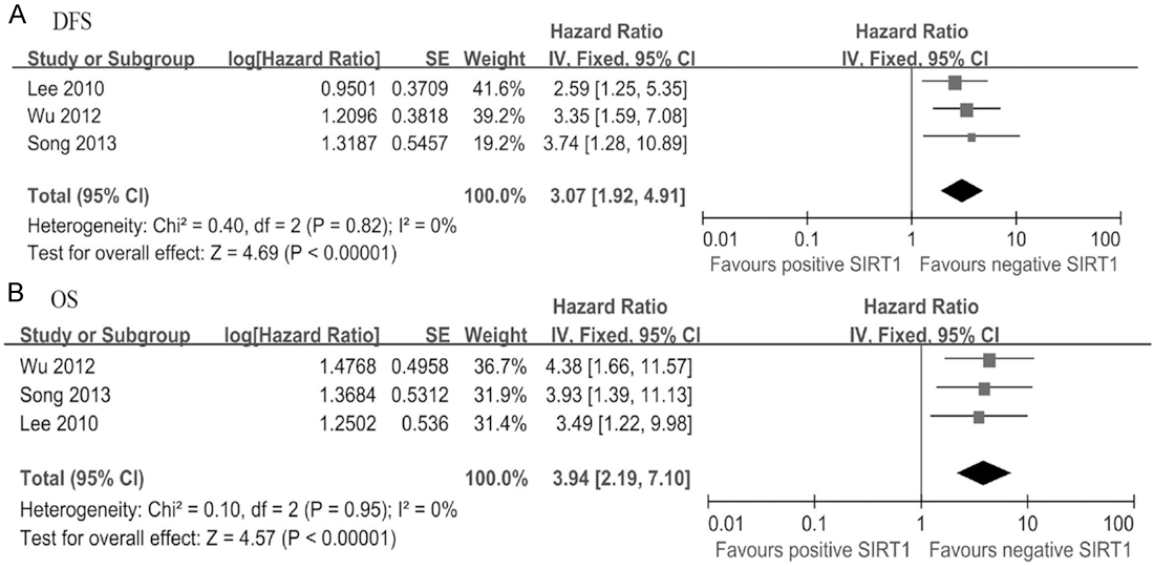

Figure 3. Forest plots of associations between SIRT1 expression with DFS (A) and OS (B) in breast cancer patients.

studies, SIRT1 expression was scored as positive if any part of the nucleus was stained [15, 16, 20]. In the other studies [9, 14, 21], a cutoff value of SIRT1 expression was determined by multiplying the percentage and intensity scores. Because of different cutoff values, we used the SIRT1 expression data of the original articles. HRs and 95% CIs for DFS and OS were directly obtained from the original articles or calculated according to the method of Parmar [22].

The relationship of SIRT1 expression to the clinical parameters of the 604 patients in the 6 studies is summarized in **Table 2**. SIRT1 expression significantly correlated with TNM stage ($P < 0.01$, OR = 2.92, 95% CI: 1.84-4.63) and marginally correlated with LNM ($P = 0.05$, OR =

3.22, 95% CI: 0.98-10.57) (**Figure 2**). There was no statistically significant association between SIRT1 expression and tumor size, histological grade, or ER, PR, or HER-2 status. Because of significant heterogeneity, the random-effects model was used for the combined analyses of tumor size, LNM, grade, and PR status (P_h = 0.05, I^2 = 61%; P_h < 0.001, I^2 = 88%; P_h = 0.02, I^2 = 66%; and P_h < 0.001, I^2 = 79%, respectively). Because of no evident heterogeneity, the fixed-effects model was used for analysis of TNM, HER-2 status, and ER status ((P_h = 0.32, I^2 = 14%; P_h = 0.26, I^2 = 24%; and P_h = 0.13, I^2 = 43%, respectively).

Three studies involving 346 patients investigated the association of SIRT1 with prognosis in accordance with our criteria. Overall, there was a statistically significant correlation between reduced survival (DFS and OS) and SIRT1 expression (DFS: P < 0.001, HR = 3.07, 95% CI: 1.92-4.91; OS: P < 0.001, HR = 3.94, 95% CI: 2.19-7.10) (**Figure 3**). No significant heterogeneity was observed among these studies for DFS or OS (DFS: P_h = 0.82, I^2 = 0%; OS: P_h = 0.95, I^2 = 0%); therefore, a fixed-effects model was used in our analyses.

Publication bias

Begg's funnel plots and Egger's tests were carried out to assess the publication bias of the included literature. Begg's funnel plots revealed no evidence of significant asymmetry (TNM: P = 0.821; LNM: P = 0.573; DFS: P = 0.515; OS: P = 0.272) nor did Egger's test (TNM: P = 0.851; LNM: P = 0.247; DFS: P = 0.117; OS: P = 0.117) (**Figure 4**).

Discussion

To our knowledge, we are the first to provide compelling evidence of a significant correlation between SIRT1 expression and high TNM stage, LNM, and short DFS and OS in breast cancer. On the basis of our meta-analysis of 604 patients from 6 studies, we suggest that SIRT1 has prognostic value as an indicator of poor survival in patients with breast cancer.

Accumulating evidence shows that epigenetic alterations including histone modifications are critical for breast carcinogenesis [23]. Recent studies suggest that histone deacetylase SIRT1 exhibits a dual effect on the initiation and progression of breast carcinomas [24, 25], but the underlying mechanisms are extremely complicated and remain largely unknown. In support of an oncogenic role of SIRT1 in breast cancer, Sung and co-workers [9] recently observed higher levels of SIRT1 in breast cancer cells than in normal cells. Conversely, Wang and co-workers [26] found that SIRT1 expression was lower in breast cancer tissues than in normal tissues and that restoration of SIRT1 levels in breast cancer cells inhibited their proliferation and tumor formation. Therefore, the role of SIRT1 in the initiation and progression of breast cancer is unclear.

In the present study, we investigated the relationship between SIRT1 expression and clinicopathological parameters via meta-analysis. We found that SIRT1 expression was more frequently elevated in patients with a high compared with a low TNM stage, suggesting that SIRT1 expression is strongly correlated with aggressive biological behavior. In agreement, other studies reported [14, 15] that SIRT1 expression was significantly associated with TNM stage. We also found that SIRT1 expression was marginally correlated with lymph node metastasis. In agreement, Song and co-workers [15] reported that SIRT1 expression was higher in patients with lymph node metastasis. Therefore, findings from previous studies and our meta-analysis suggest that SIRT1 may serve as a biomarker for predicting tumor cell aggressiveness and metastasis in breast cancer.

The prognostic significance of SIRT1 expression for patients with breast cancer remains controversial. Wu and co-workers [14] found that patients with SIRT1 expression had significantly shorter DFS and OS times, which suggested that SIRT1 may be a potential biomarker of poor prognosis in breast cancer patients. In agreement, SIRT1 expression correlated significantly with distant metastatic relapse and shorter relapse-free survival and OS times in Korean patients with breast cancer [16]. Our meta-analysis also significantly correlated SIRT1 expression with reduced survival rates (both DFS and OS) in breast cancer patients. On the other hand, SIRT1 expression was not associated with the survival of breast cancer patients in the study of Sung and co-workers [9]. The discrepancies in the results of individual studies may reflect small sample sizes and perhaps a dual role of SIRT1 in breast cancer.

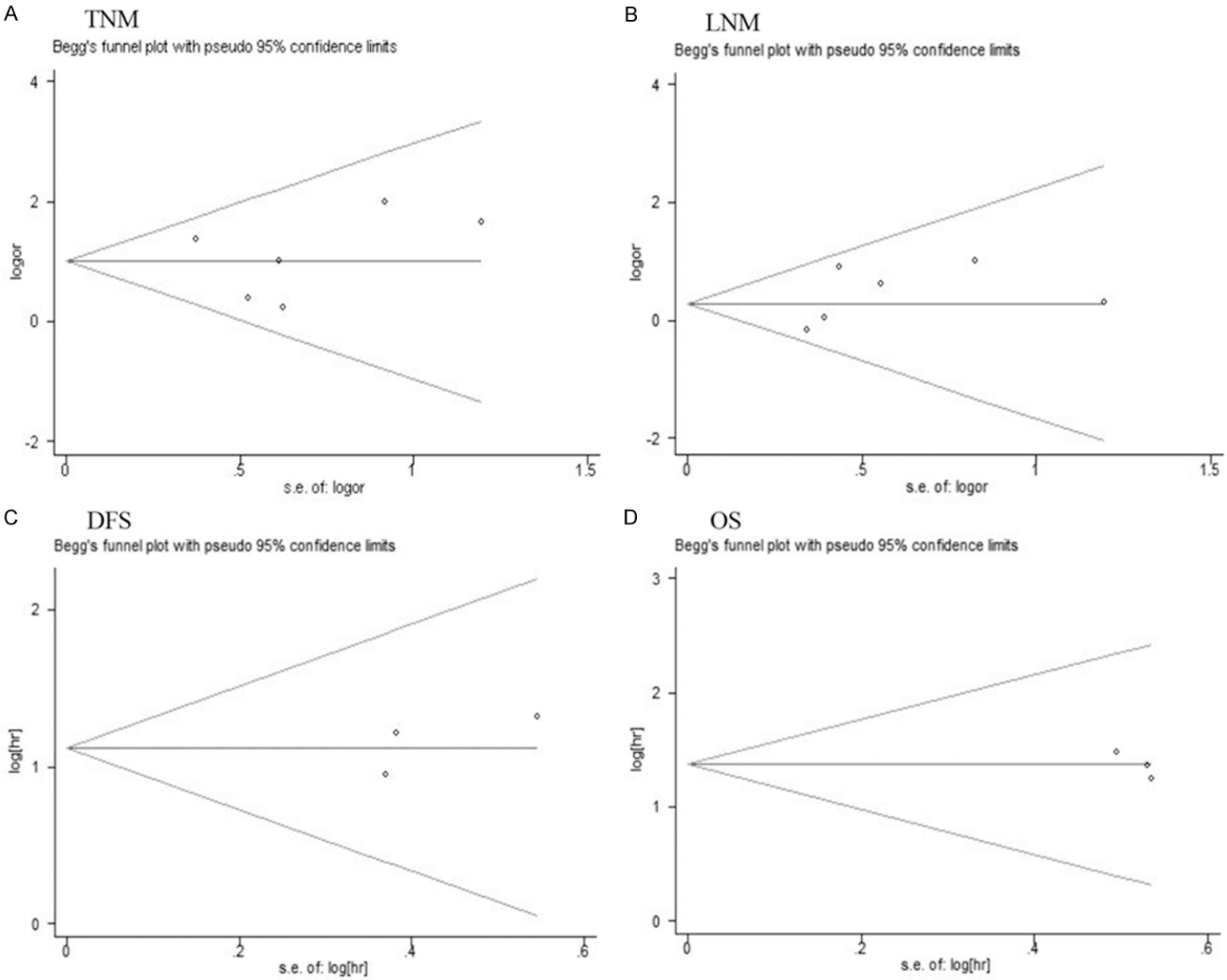

Figure 4. Funnel plots for the evaluation of potential publication bias in the impact of SIRT1 expression on TNM (A), LNM (B), DFS (C) and OS (D) in breast cancer patients. The Egger test for publication bias was not significant for TNM (P = 0.821), LNM (P = 0.573), DFS (P = 0.515) and OS (P = 0.272).

Overall, the most recent findings [14, 16] and our results suggest that SIRT1 may serve as a useful predictor of poor prognosis in breast cancer. The mechanism by which SIRT1 might promote the initiation or progression of breast cancer remains largely unknown, and further mechanistic studies are needed.

Several limitations of our study need to be pointed out. First, the results of our meta-analysis are unlikely to achieve sufficient statistical power to conclusively associate SIRT1 expression with specific parameters because of the lack of necessary data. Second, the reliability of our results may be limited by the information provided in the studies examined. Third, the definition of SIRT1 positivity varied between the selected studies, and variability in assessments may lead to potential bias. Fourth, the studies included in our analysis were all performed in Asia, and the results obtained may not be representative of breast cancers in other parts of the world. Despite the above limitations, our study is the first meta-analysis of the association of SIRT1 expression with the clinicopathological characteristics and prognosis of breast cancer. Importantly, it utilized a statistical approach to combine the results of multiple studies, achieving objectivity on the basis of strict inclusion and exclusion criteria.

In conclusion, our study is the first meta-analysis showing an association of SIRT1 expression with high TNM stage and poor survival in patients with breast cancer. More large-scale, multi-center, and well-matched cohort investigations are warranted to clarify the role of SIRT1 in breast cancer prognosis and clinical applications.

Acknowledgements

We thank all the authors whose articles were included in our meta-analysis.

Disclosure of conflict of interest

None.

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