Breast Cancer Res Treat (2013) 142:619–627
DOI 10.1007/s10549-013-2774-9

EPIDEMIOLOGY

Promoter methylation of *BRCA1* in the prognosis of breast cancer: a meta-analysis

Liang Wu · Fengliang Wang · Ruobing Xu · Simin Zhang · Xianzhen Peng · Yan Feng · Jianming Wang · Cheng Lu

Received: 11 September 2013 / Accepted: 10 November 2013 / Published online: 21 November 2013

Abstract The inactivation of *BRCA1* by epigenetic alterations is a critical event in breast tumorigenesis, which may potentially be used as a prognostic marker for patients with breast cancer. The present study systematically reviewed the promoter methylation of *BRCA1* and its relationship to the clinical outcomes of breast cancer patients. We performed a meta-analysis following the PRISMA guideline. Relevant articles were identified by searching PubMed, Web of Science and Embase database until August 2013. The pooled hazard ratio (HR) and 95 % confidence interval (CI) were applied to estimate the effect of *BRCA1* methylation. Random or fixed effect model was chosen based on the heterogeneity analysis. A total of 3,205 patients from nine eligible studies were included in the meta-analysis. *BRCA1* methylation was found to be significantly correlated with a poor overall survival of breast cancer, with the combined HR (95 % CI) of 2.02 (1.35–3.03). After adjusting for potential confounders using the Cox regression model, the pooled HR (95 % CI) of *BRCA1* methylation on patients' overall survival was 1.38 (1.04–1.84). If we used the disease-free survival as the outcome, the combined HR (95 % CI) was 2.89 (1.73–4.83) for univariate analysis and 3.92 (95 % CI 1.49–10.32) for multivariate analysis, respectively. Subgroup analysis of specimen types revealed that the pooled HR (95 % CI) for overall survival was 1.48 (1.22–1.81) when using formalin-fixed paraffin-embedded (FFPE) specimen and 1.38 (0.16–11.84) when using fresh frozen tissues. As for the disease-free survival, the pooled HR (95 % CI) was 2.47 (1.33–4.58) when using FFPE specimen and 2.78 (1.47–5.28) when using fresh frozen tissues. As a conclusion, the present meta-analysis provides evidence that *BRCA1* methylation is associated with a poor survival of breast cancer patients. Our findings underscore the clinical relevance of aberrant epigenetic alteration as a promising biomarker for the prognosis of human cancers.

Keywords Breast cancer · BRCA1 · Methylation · Prognosis · Survival · Meta-analysis

Introduction

Breast cancer (BC) has been ranked as the most frequent cancer among women, with an estimated 1.38 million new cases diagnosed in 2008 (23 % of all cancers) [1, 2]. It represents a heterogeneous group of tumors with varied biologic and morphologic features, behaviors, and responses to treatment, posing challenges for clinicians regarding the choice of optimum adjuvant therapy [3]. Traditionally, tumor size, histologic grade, lymph node metastasis, endocrine receptor status, and human epidermal growth factor receptor 2 (HER2) expression are widely used as prognostic factors for patients with breast cancer. There have been increasing concerns that these variables are limited in their ability to capture the diversity of clinical behaviors and insufficient to tailor individualized therapy [4]. The application of specific molecular markers

Liang Wu and Fengliang Wang have contributed equally to this work.

L. Wu · R. Xu · S. Zhang · X. Peng · Y. Feng · J. Wang (✉)
Department of Epidemiology and Biostatistics, School of Public Health, Nanjing Medical University, Nanjing, People's Republic of China
e-mail: jmwang@njmu.edu.cn

F. Wang · C. Lu (✉)
Department of Breast, Nanjing Maternity and Child Health Hospital of Nanjing Medical University, Nanjing, People's Republic of China
e-mail: lucheng66@126.com

is powerful to help make therapeutic decisions at the individual level with aims to improve patients' management and prolong their survival time.

Epigenetic alteration is one of the most common molecular changes in the development of human cancers [5, 6]. Major epigenetic mechanisms include aberrant DNA methylation, changes of histone and chromatin structure by posttranslational modification of histone proteins, and alterations in the expression of microRNAs [7]. Aberrant DNA methylation can alter the normal gene expression, genomic structure, as well as genetic stability [8]. It is well established that widespread changes of DNA methylation occur during carcinogenesis and tumor progression [9]. Different from other biomarkers in breast cancer which are usually based on gene expression, DNA methylation has been identified with independent prognostic value and can be used in tailoring treatment to patients who are receiving uniform therapy regimens today [10].

BRCA1 (breast cancer 1, early onset; Gene ID: 672) tumor suppressor gene maps to chromosome 17q12-21 and encodes a multifunctional protein involved in DNA repair, control of cell-cycle checkpoints, protein ubiquitinylation, and chromatin remodeling [11, 12]. It has become clear that the inactivation of *BRCA1* by epigenetic alterations is a critical event in breast tumorigenesis. Researches have observed aberrant methylation of *BRCA1* in association with relatively poor clinical outcomes [13, 14]. It may potentially be used as a prognostic marker relating to overall survival (OS) and disease-free survival (DFS) of patients with breast cancer [14–16].

However, due to the different sensitivities and intra-/inter-assay coefficients of variation of methods, the reported proportion of *BRCA1* methylation is highly variable, and its prognostic value remains controversial [17]. Therefore, we performed a meta-analysis to clarify the role of *BRCA1* methylation in the prognosis of patients with breast cancer.

Methods

Data collection

We performed this meta-analysis according to the guidelines of Preferred Reporting Items for Systematics Reviews and Meta-Analyses (PRISMA) set by the PRISMA Group [18]. We searched for published articles in PubMed, Web of Science, and Embase databases (last search updated on August 28, 2013) using the following terms and their various combinations: "*BRCA1*," "breast cancer," "methylation," and "prognostic" or "survival." Additional studies were also identified via the references listed in the articles. Studies selected for meta-analysis had to meet the following criteria: (1) provided DFS and/or OS to evaluate the role of methylation status of *BRCA1* in the prognosis of breast cancer; (2) hazard ratio (HR) with its 95 % confidence interval (CI) was reported or could be calculated from the data presented in the article; (3) DNA methylation was detected from the whole blood, plasma, serum, or tissues; (4) studies with full text articles. Exclusion criteria included: (1) data from reviews, animal or cell line studies; (2) studies published in any language other than English.

Data extraction

Two graduate students independently read the articles and extracted data with a data extraction form, which included the name of the first author, year of publication, number of study subjects, proportion of *BRCA1* methylation, disease stage, tumor grade, methylation detection method, and effects on clinical outcomes (OS and DFS). OS referred to the time of initial diagnosis to the death of the breast cancer patient. DFS was defined as the time between initial diagnosis and disease recurrence or the last follow-up assessment.

Statistical analysis

We used the HR and 95 % CI to estimate the effect of *BRCA1* methylation on the prognostic value among patients with breast cancer. The adjusted HRs (95 % CIs) for OS and DFS were calculated using data drawn from the Cox regression model. When HRs (95 % CIs) were not provided directly in the article, we contacted the authors for more information or estimated them by means of the methods provided by Tierney et al. [19]. We used the Cochran's Q test (significant cutoff point: $P = 0.10$) and I^2 ($I^2 < 25$ %, no heterogeneity; $I^2 = 25$–50 %, moderate heterogeneity; and $I^2 > 50$ %, strong heterogeneity) to test the heterogeneity between studies [20, 21]. Results without significant heterogeneity were pooled using the fixed effect model [22]. Otherwise, we used the random effect model [23]. Galbraith plot was used to detect the potential sources of heterogeneity from the meta-analysis [24]. A sensitivity analysis was performed by removing one study each time to assess the stability of the results [25]. The publication bias was assessed by funnel plot and Egger's test [26]. Analyses were carried out using STATA 11.0 software (Stata Corporation, College Station, TX, USA).

Results

Characteristics of studies

By the initial search, fifty-two potentially relevant articles were identified. Then, we excluded nine articles because of

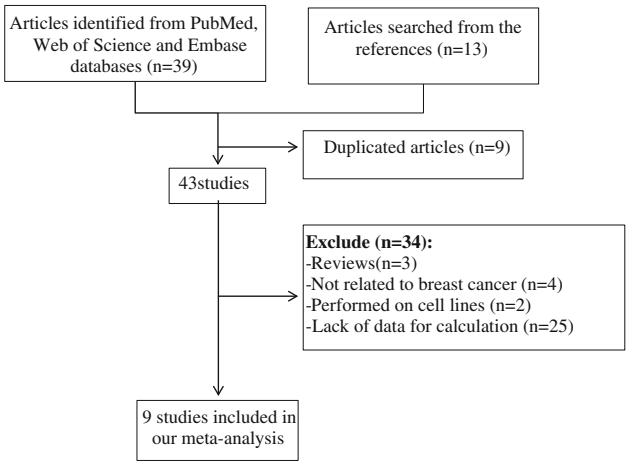

Fig. 1 Flow diagram of the selection procedure of studies

duplicated publication. After carefully reading the articles, 34 were excluded (three articles were reviews; four articles were not related to breast cancer; two articles were performed on cell lines; twenty-five articles did not present data about survival outcomes). Finally, nine articles with 3,205 study subjects (3,305 samples) were included in this meta-analysis (Fig. 1). The characteristics of these studies are listed in Table 1. The sample size for each study ranged from 78 to 1,163, with a median sample size of 135 patients. The frequency of *BRCA1* methylation varied from 17.0 to 59.2 %, with the average proportion of 35.76 %. These studies originated from six countries or regions (including the mainland of China, Taiwan, Bulgaria, India, USA, and Tunisia). All studies used the methylation-specific PCR (MSP) to measure the methylation status of the *BRCA1* gene. Among them, three studies used fresh frozen tissues, four used formalin-fixed paraffin-embedded (FFPE) specimens, one used peripheral blood samples, and one used both FFPE specimen and peripheral blood sample. Due to insufficient data, HRs on OS could be extracted from eight studies for univariate analysis and five studies for multivariate analysis. According to DFS analysis, there were six studies with available data for univariate analysis and five studies with available data for multivariate analysis.

Methylation of *BRCA1* and overall survival of patients with breast cancer

Considering the significant heterogeneity between studies ($P = 0.005, I^2 = 63.8$ %), we used the random effect model to estimate the combined effect of *BRCA1* methylation. As shown in Table 2, *BRCA1* methylation was significantly related to poor OS of breast cancer, with the combined HR (95 % CI) of 2.02 (1.35–3.03) (Fig. 2). After considering potential confounders by adjusting for age, menopausal status, tumor size, tumor stage, and estrogen receptor (ER)α

Table 1 The main characteristics of eligible studies

First author	Year	Country/region	Methods	No. of patients	Methylation (%)	Stage	Grade	Sample type	OS		DFS	
									Univariate analysis HR (95 % CI)	Multivariate analysis HR (95 % CI)	Univariate analysis HR (95 % CI)	Multivariate analysis HR (95 % CI)
Xu [27]	2013	China	MSP	1,163	25.3	N/A	I–III	FFPE tissue	1.29 (0.96–1.73)	N/A	1.34 (1.06–1.71)	N/A
Hsu [16]	2013	Taiwan	MSP	139	56.1	I–IIA	I–III	Fresh frozen tissues	4.23 (1.08–16.52)	16.38 (1.37–195.45)	2.43 (1.11–5.31)	12.19 (2.29–64.75)
Krasteva [37]	2012	Bulgaria	MSP	135	17.0	N/A	I–III	Fresh frozen tissues	0.47 (0.14–1.54)	0.91 (0.24–3.41)	N/A	N/A
Sharma [28]	2010	India	MSP	100	27.0	I–III	N/A	FFPE tissue	5.32 (1.17–24.06)	N/A	9.0 (3.4–23.8)	7.5 (2.8–20.4)
Sharma [28]	2010	India	MSP	100	25.0	I–III	N/A	Serum	5.27 (1.12–24.79)	N/A	8.11 (2.44–26.94)	N/A
Sharma [29]	2009	India	MSP	101	26.7	I–III	N/A	FFPE tissue	5.06 (1.58–16.22)	2.12 (0.47–9.63)	3.88 (2.05–7.34)	2.03 (0.96–4.29)
Chen [13]	2009	China	MSP	536	25.4	I–III	N/A	FFPE tissue	1.56 (1.02–2.37)	1.27 (0.81–1.99)	1.45 (1.01–2.09)	1.23 (0.84–1.80)
Xu [14]	2009	USA	MSP	851	59.2	N/A	N/A	FFPE tissue	1.45 (0.99–2.11)	1.40 (0.94–2.08)	N/A	N/A
Karray-Chouayekh [30]	2009	Tunisia	MSP	78	44.8	N/A	N/A	Fresh frozen tissues	N/A	N/A	3.67 (1.20–11.18)	20.7 (1.7–251.5)
Feng [31]	2008	China	MSP	102	32.4	N/A	I–III	Serum	6.4 (2.0–20.5)	N/A	N/A	N/A

MSP methylation-specific PCR, *N/A* not available, *FFPE* formalin-fixed paraffin-embedded, *OS* overall survival, *DFS* disease-free survival, *HR (95 % CI)* hazard ratio (95 % confidence interval)

Table 2 Evaluation of *BRCA1* methylation in association with OS or DFS of breast cancer patients

Factors	No. of studies/ cases	HR (95 % CI)	Heterogeneity test		
			χ^2	*P*	I^2 (%)
Overall survival (*OS*)					
All studies					
Univariate analysis	8/3227	2.02 (1.35–3.03)	22.12	0.005	63.8
Multivariate analysis	5/1762	1.38 (1.04–1.84)	4.65	0.325	13.9
Subgroup analysis by sample types					
Tissue samples					
Univariate analysis	7/3025	1.65 (1.14–2.38)	13.37	0.033	56.3
Multivariate analysis	5/1762	1.38 (1.04–1.84)	4.65	0.325	13.9
FFPE tissues					
Univariate analysis	5/2751	1.48 (1.22–1.81)	7.94	0.094	49.6
Multivariate analysis	3/1488	1.37 (1.02–1.83)	0.44	0.802	0.0
Fresh frozen tissues					
Univariate analysis	2/247	1.38 (0.16–11.84)	5.62	0.018	82.2
Multivariate analysis	2/247	3.17 (0.19–52.04)	4.06	0.044	75.3
Serum					
Univariate analysis	2/202	5.97 (2.35–15.13)	0.04	0.844	0.0
Multivariate analysis	–	–	–	–	–
Disease-free survival (*DFS*)					
All studies					
Univariate analysis	6/2217	2.89 (1.73–4.83)	31.31	0.000	80.8
Multivariate analysis	5/954	3.92 (1.49–10.32)	20.57	0.000	80.6
Subgroup analysis by sample types					
FFPE tissue					
Univariate analysis	4/1900	2.47 (1.33–4.58)	21.91	0.000	86.3
Multivariate analysis	3/737	2.43 (0.94–6.27)	11.50	0.003	82.6
Fresh frozen tissues					
Univariate analysis	2/217	2.78 (1.47–5.28)	0.35	0.553	0.0
Multivariate analysis	2/217	14.36 (3.58–57.58)	0.12	0.730	0.0

and progesterone receptor (PR) status, the pooled HR (95 % CI) for *BRCA1* methylation on OS was 1.38 (1.04–1.84) (Fig. 3). We further performed a subgroup analysis by considering the type of samples used for detecting DNA methylation. The combined HRs (95 % CIs) by univariate analysis were 1.48 (1.22–1.81) for FFPE specimen and 1.38 (0.16–11.84) for fresh frozen tissues. The combined HRs (95 % CIs) by multivariate analysis were 1.37 (1.02–1.83) for FFPE specimen and 3.17 (0.19–52.04) for fresh frozen tissues. The effect was stronger for serum samples (HR: 5.97, 95 % CI 2.35–15.13) as compared with tissue samples (HR: 1.65, 95 % CI 1.14–2.38).

Methylation of *BRCA1* and disease-free survival of patients with breast cancer

Six studies were eligible for meta-analysis of *BRCA1* methylation of the DFS, including 6 studies for univariate analysis [13, 16, 27–30] and five studies for multivariate analysis [13, 16, 28–30] (Figs. 4, 5). The combined HR was 2.89 (95 % CI 1.73–4.83) for univariate analysis and 3.92 (95 % CI 1.49–10.32) for multivariate analysis. In addition, a subset of four studies [13, 27–29] (1,900 patients) reported the DFS by means of FFPE specimen, and a subset of two studies [16, 30] (217 patients) reported the DFS by means of fresh tissue samples. For studies using FFPE specimen, the pooled HR was 2.47 (95 % CI 1.33–4.58) for univariate analysis and 2.43 (95 % CI 0.94–6.27) for multivariate analysis. For studies using fresh frozen tissues, the pooled HR was 2.78 (95 % CI 1.47–5.28) for univariate analysis and 14.36 (95 % CI 3.58–57.58) for multivariate analysis.

Sensitivity analyses and publication bias assessment

We used the Galbraith plot to explore the heterogeneity and to check if individual study affected the results. The visualization of the funnel plots for OS univariate analysis

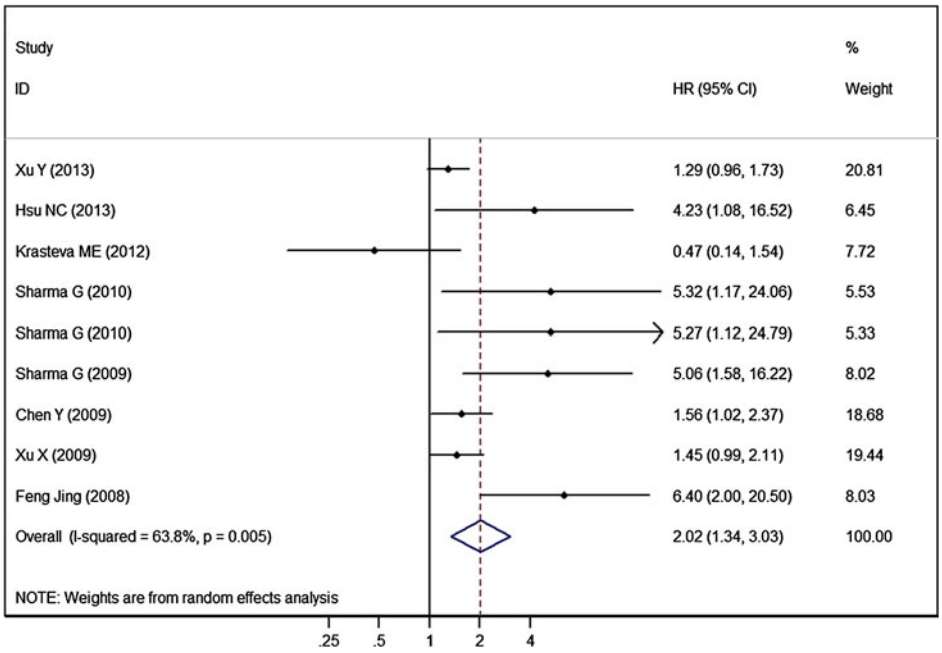

Fig. 2 Forest plot showing the association between *BRCA1* methylation and OS of breast cancer using univariate analysis

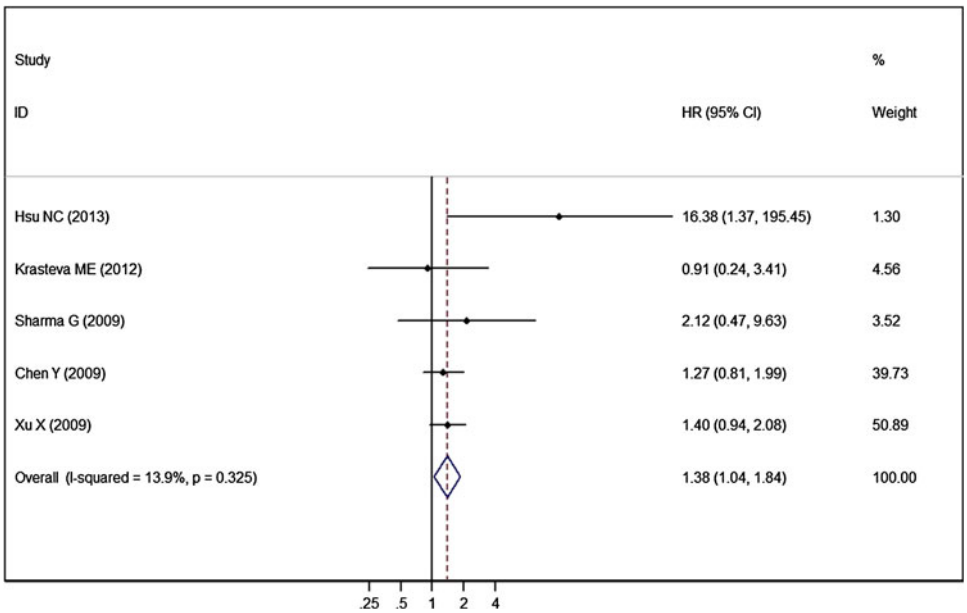

Fig. 3 Forest plot showing the association between *BRCA1* methylation and OS of breast cancer using multivariate analysis

showed that the two publications, Feng et al. [31] and Sharma et al. [29], accounted for the observed heterogeneity. When we moved out these two studies, the heterogeneity disappeared ($P = 0.062$, $I^2 = 50$ %). However, on the Galbraith plot of DFS, only 4 studies were located within the 95 % bounds (the zone of two outer parallel lines drawn at two units over and below the regression) from the standardized mean lnHR.

We used the leave-one-out sensitivity analyses by removing one study per time to check if individual study influenced the results. The result pattern was not obviously impacted by any single study. We then used the Egger's linear regression model and Begg's funnel plot to test the publication bias (Figs. 6, 7). For OS analysis, the Begg's test $P = 0.348$ and the Egger's test $P = 0.049$. For DFS analysis, the Begg's test $P = 0.133$ and the Egger's test $P = 0.004$.

Discussion

Evolved management of breast cancer and availability of various treatment options have led to a significant decline of

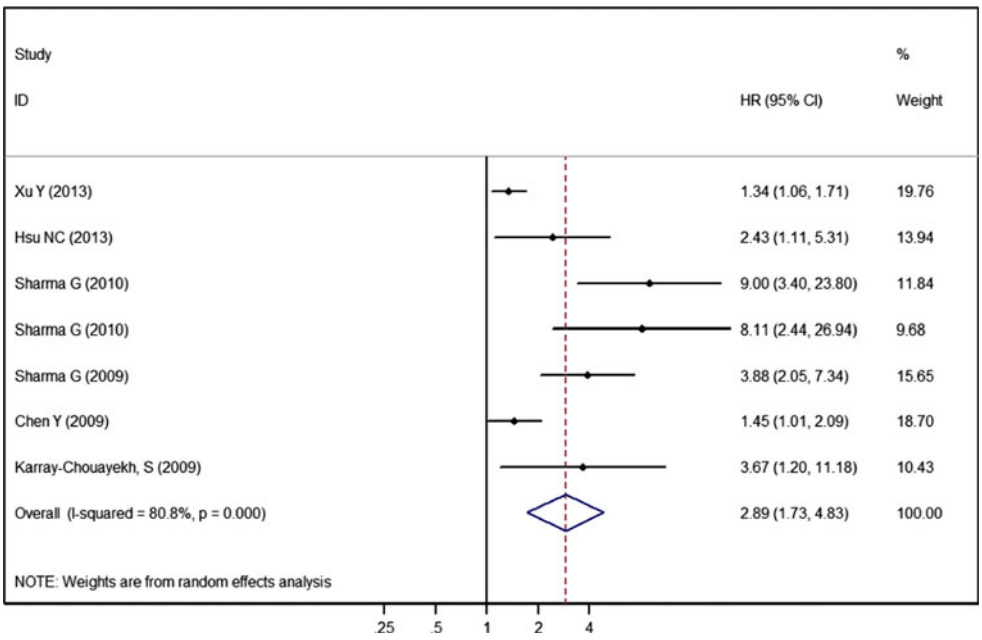

Fig. 4 Forest plot showing the association between *BRCA1* methylation and DFS of breast cancer using univariate analysis

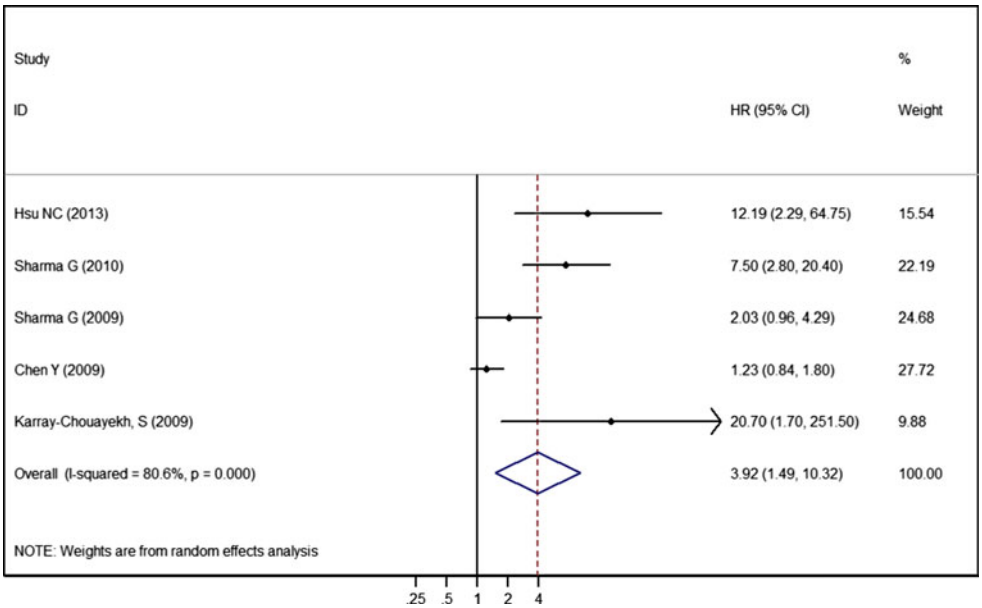

Fig. 5 Forest plot showing the association between *BRCA1* methylation and DFS of breast cancer using multivariate analysis

cancer mortality [32]. Adjuvant systemic treatment in breast cancer includes multiple-chemotherapy regimens, and each therapeutic option has its own specific benefit and adverse effect. A significant proportion of patients who have poor prognosis will develop recurrence even if receiving adjuvant chemotherapy [33]. This necessitates a need for more sensitive and specific prognostic indicators. Epigenetic alteration is one of the most common molecular changes in human cancers [15]. Hypermethylation of gene promoters occurs early in the development of tumors, which may provide independent prognostic information and have the ability to reflect multiple aspects of diseases [34].

The *BRCA1* gene was cloned in 1994 as one of the genes that conferred genetic predisposition to early onset of breast and ovarian cancer [35]. Despite being implicated in many important cellular pathways, including DNA repair and regulation of transcription, the exact mechanism by which inactivation of *BRCA1* might lead to malignant transformation of cells remains unclear [35]. Reports have suggested that tumors with genetic defects in *BRCA1* are more sensitive to growth inhibition and chromosomal damage upon platinum-based chemotherapy [36]. However, as few breast cancer patients are carrying *BRCA1* mutations, CpG island promoter hypermethylation-

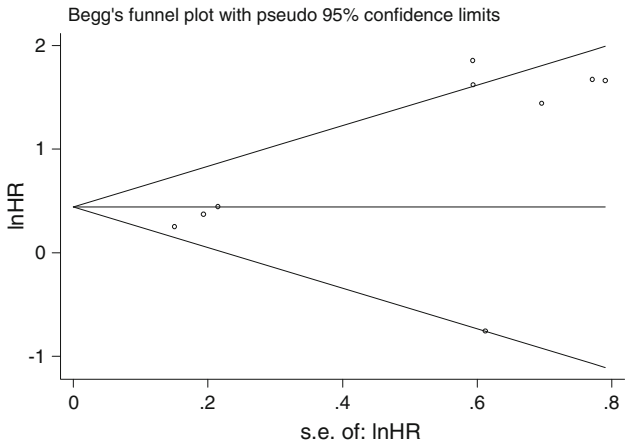

Fig. 6 Begg's funnel plot for OS of breast cancer

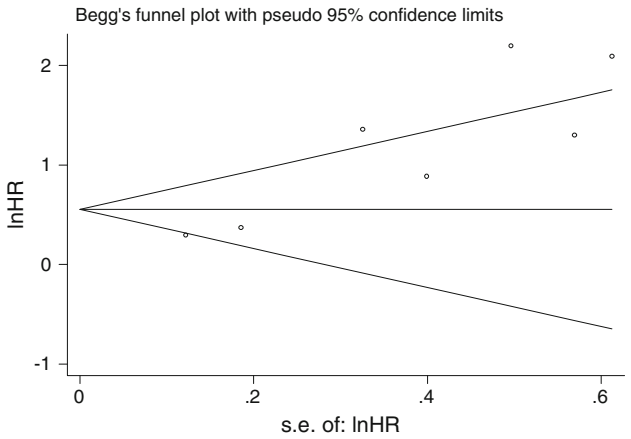

Fig. 7 Begg's funnel plot for DFS of breast cancer

associated silencing of *BRCA1* is also being believed to play critical roles in inactivation of the *BRCA1* gene and enhance the sensitivity to platinum-derived drugs to the same extent as *BRCA1* mutations [36]. Previous studies have underscored the potential utility of aberrant DNA methylation of *BRCA1* as a promising biomarker for diagnosis and prognosis of breast cancer [28].

To summarize the current research progress of the *BRCA1* methylation on the survival of breast cancer patients, we present this meta-analysis by including nine articles and 3,205 patients. Findings of this meta-analysis revealed that *BRCA1* is a significant predictor for both OS and DFS of patients with breast cancer, even after adjusting for other prognostic factors. However, one study in Bulgaria reported that breast cancer patients with hypermethylation in the promoter of *BRCA1* gene exhibited favorable clinical outcomes [37]. In this study, the authors investigated the methylation in the promoter region of *BRCA1* and its correlation with clinico-pathological and molecular characteristics in a group of 135 breast cancer patients. MSP was applied to determine methylation status of tumor samples. The presence of hypermethylation was weakly associated with better OS ($P = 0.2$) with a HR of 0.47 (95 % CI 0.14–1.54). Patients with hypermethylation in *BRCA1* exhibited more favorable clinical status as their tumors were smaller, lacked p53 mutations, and were of lobular type [37].

Samples used for DNA methylation measurement included fresh tissues, paraffin-embedded tissues, body fluids, whole blood, or cell-free DNA in peripheral blood. When stratified analysis was conducted on the sample types, we found that both FFPE and fresh frozen tissue samples had significant associations between *BRCA1* methylation status and DFS. However, for OS analysis, these two types of samples did not show similar results. It was reported that the methylation status in paraffin-embedded tumor tissues might be potentially altered by the resection to fixation or the process of fixation itself, which may cause heterogeneity of studies [38, 39]. There is also a significant heterogeneity when using tissue samples ($I^2 = 56.3$ %, $P = 0.033$), but not for serum samples ($I^2 = 0.0$ %, $P = 0.844$), suggesting the methylation status in serum may be a more sensitive and specific prognostic biomarker of breast cancer. There are two types of DNA present in circulating blood: DNA associated with lymphocytes and the so-called cell-free circulating DNA in either plasma or serum [40]. Several studies have demonstrated that cancer patients have abnormally high levels of serum tumor-specific DNA alterations, with more than 90 % of the total circulating cell-free DNA derived from tumor tissues [41, 42]. Increased plasma cell-free DNA extracted from cancer patients had all the characteristics of tumor DNA. In response to treatment, methylation patterns in cell-free DNA become more comparable to those of healthy controls, suggesting that methylation in cell-free DNA may be useful for treatment monitoring [17].

There are several limitations in this study. First, the number of relevant studies eligible for this meta-analysis was relatively small. Most studies were carried out in Asian populations. Different patient selection criteria, chemotherapeutic protocol, and follow-up period were the possible explanations for the heterogeneity. Second, though we estimated the outcomes by means of the methods provided by Tierney et al. [19] if the original article didn't provide necessary data, the outcomes calculated from Kaplan–Meier curve or log-rank test may have produced some imprecision. Third, although the Egger's test did not reach the statistically significant level, publication bias may still influence the results and leads to false-positive association. Asymmetrical appearance of the funnel plot could be caused by inflated estimates by a flawed methodological design in smaller studies and/or a lack of publication of trials with opposite results. Although funnel plot

asymmetry is often interpreted to indicate publication bias, it is important to consider that this asymmetry may also be due to other sources of bias.

Besides *BRCA1*, other genes like *BRCA2*, *APC*, *P16INK4a,* and *RASSF1A* have also been associated with tumorigenesis and have been suggested to be included in the models that evaluate individual breast cancer risk [43]. Both *BRCA1* and *BRCA2* are involved in maintenance of genome stability, specifically the homologous recombination pathway for double-strand DNA repair. Preliminary evidence suggests that *BRCA2* confers a high risk of breast cancer but, unlike *BRCA1*, does not confer a substantially elevated risk of ovarian cancer [44]. Correlations were found between *BRCA1* and *BRCA2* hypermethylation and decrease in their mRNA expression [45]. However, studies of the role of *BRCA2* methylation on the prognosis of patients with breast cancer are limited. Thus, we only included *BRCA1* in this meta-analysis. Current efforts in discovery, validation and qualification of biomarkers of breast cancer will offer considerable promise in the future to develop more accurate breast cancer risk assessment [46]. Based on the observation of the combined effects of promoter methylation of tumor suppressor genes, it suggests that multiple epigenetic changes may be included in prognosis models of breast cancer.

Conclusion

The present meta-analysis provides evidence that *BRCA1* methylation is associated with the poor survival of breast cancer patients. Our findings underscore the clinical relevance of aberrant epigenetic alteration as a promising biomarker for the prognosis of human cancers.

Acknowledgments This study is supported by the National Natural Science Foundation of China (81172268, 81172501), Key University Science Research Project of Jiangsu Province (12KJA330001), and Priority Academic Program Development of Jiangsu Higher Education Institutions (PAPD).

Conflict of interest The authors declare that they have no competing interests.

References

1. Ferlay J, Shin HR, Bray F, Forman D, Mathers C, Parkin DM (2010) Estimates of worldwide burden of cancer in 2008: GLOBOCAN 2008. Int J Cancer J 127(12):2893–2917. doi:10.1002/ijc.25516
2. Jemal A, Bray F, Center MM, Ferlay J, Ward E, Forman D (2011) Global cancer statistics. CA Cancer J Clin 61(2):69–90. doi:10.3322/caac.20107
3. Mukherjee A, Rakha EA (2012) Integrating breast cancer genetics into clinical practice. Womens Health (Lond Engl) 8(1):99–112. doi:10.2217/whe.11.81
4. Rakha EA, Reis-Filho JS, Baehner F, Dabbs DJ, Decker T, Eusebi V, Fox SB, Ichihara S, Jacquemier J, Lakhani SR, Palacios J, Richardson AL, Schnitt SJ, Schmitt FC, Tan PH, Tse GM, Badve S, Ellis IO (2010) Breast cancer prognostic classification in the molecular era: the role of histological grade. Breast Cancer Res 12(4):207. doi:10.1186/bcr2607
5. Baylin SB, Belinsky SA, Herman JG (2000) Aberrant methylation of gene promoters in cancer—concepts, misconcepts, and promise. J Natl Cancer Inst 92(18):1460–1461
6. Jones PA, Laird PW (1999) Cancer epigenetics comes of age. Nat Genet 21(2):163–167. doi:10.1038/5947
7. Toh Y, Egashira A, Yamamoto M (2013) Epigenetic alterations and their clinical implications in esophageal squamous cell carcinoma. Gen Thorac Cardiovasc Surg 61(5):262–269. doi:10.1007/s11748-013-0235-3
8. Fang F, Turcan S, Rimner A, Kaufman A, Giri D, Morris LG, Shen R, Seshan V, Mo Q, Heguy A, Baylin SB, Ahuja N, Viale A, Massague J, Norton L, Vahdat LT, Moynahan ME, Chan TA (2011) Breast cancer methylomes establish an epigenomic foundation for metastasis. Sci Transl Med 3 (75):75ra25. doi:10.1126/scitranslmed.3001875
9. Jones PA, Baylin SB (2007) The epigenomics of cancer. Cell 128(4):683–692. doi:10.1016/j.cell.2007.01.029
10. Cheol Kim D, Thorat MA, Lee MR, Cho SH, Vasiljevic N, Scibior-Bentkowska D, Wu K, Ahmad AS, Duffy S, Cuzick JM, Lorincz AT (2012) Quantitative DNA methylation and recurrence of breast cancer: a study of 30 candidate genes. Cancer Biomark 11(2–3):75–88. doi:10.3233/CBM-2012-0266
11. Hall JM, Lee MK, Newman B, Morrow JE, Anderson LA, Huey B, King MC (1990) Linkage of early-onset familial breast cancer to chromosome 17q21. Science 250(4988):1684–1689
12. Ralhan R, Kaur J, Kreienberg R, Wiesmuller L (2007) Links between DNA double strand break repair and breast cancer: accumulating evidence from both familial and nonfamilial cases. Cancer Lett 248(1):1–17. doi:10.1016/j.canlet.2006.06.004
13. Chen Y, Zhou J, Xu Y, Li Z, Wen X, Yao L, Xie Y, Deng D (2009) BRCA1 promoter methylation associated with poor survival in Chinese patients with sporadic breast cancer. Cancer Sci 100(9):1663–1667. doi:10.1111/j.1349-7006.2009.01225.x
14. Xu X, Gammon MD, Zhang Y, Bestor TH, Zeisel SH, Wetmur JG, Wallenstein S, Bradshaw PT, Garbowski G, Teitelbaum SL, Neugut AI, Santella RM, Chen J (2009) BRCA1 promoter methylation is associated with increased mortality among women with breast cancer. Breast Cancer Res Treat 115(2):397–404. doi:10.1007/s10549-008-0075-5
15. Khan SI, Aumsuwan P, Khan IA, Walker LA, Dasmahapatra AK (2012) Epigenetic events associated with breast cancer and their prevention by dietary components targeting the epigenome. Chem Res Toxicol 25(1):61–73. doi:10.1021/tx200378c
16. Hsu NC, Huang YF, Yokoyama KK, Chu PY, Chen FM, Hou MF (2013) Methylation of BRCA1 promoter region is associated with unfavorable prognosis in women with early-stage breast cancer. PLoS One 8(2):e56256. doi:10.1371/journal.pone.0056256
17. Van De Voorde L, Speeckaert R, Van Gestel D, Bracke M, De Neve W, Delanghe J, Speeckaert M (2012) DNA methylation-based biomarkers in serum of patients with breast cancer. Mutat Res 751(2):304–325. doi:10.1016/j.mrrev.2012.06.001
18. Moher D, Liberati A, Tetzlaff J, Altman DG (2009) Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. PLoS Med 6(7):e1000097. doi:10.1371/journal.pmed.1000097
19. Tierney JF, Stewart LA, Ghersi D, Burdett S, Sydes MR (2007) Practical methods for incorporating summary time-to-event data into meta-analysis. Trials 8:16. doi:10.1186/1745-6215-8-16
20. DerSimonian R (1996) Meta-analysis in the design and monitoring of clinical trials. Stat Med 15(12):1237–1248; discussion

1249–1252. doi:10.1002/(SICI)1097-0258(19960630)15:12<1237::AID-SIM301>3.0.CO;2-N
21. Higgins JP, Thompson SG, Deeks JJ, Altman DG (2003) Measuring inconsistency in meta-analyses. BMJ 327(7414):557–560. doi:10.1136/bmj.327.7414.557
22. Mantel N, Haenszel W (1959) Statistical aspects of the analysis of data from retrospective studies of disease. J Natl Cancer Inst 22(4):719–748
23. DerSimonian R, Laird N (1986) Meta-analysis in clinical trials. Control Clin Trials 7(3):177–188
24. Bax L, Ikeda N, Fukui N, Yaju Y, Tsuruta H, Moons KG (2009) More than numbers: the power of graphs in meta-analysis. Am J Epidemiol 169(2):249–255. doi:10.1093/aje/kwn340
25. Lau J, Ioannidis JP, Schmid CH (1997) Quantitative synthesis in systematic reviews. Ann Intern Med 127(9):820–826
26. Egger M, Davey Smith G, Schneider M, Minder C (1997) Bias in meta-analysis detected by a simple, graphical test. BMJ 315(7109):629–634
27. Xu Y, Diao L, Chen Y, Liu Y, Wang C, Ouyang T, Li J, Wang T, Fan Z, Fan T, Lin B, Deng D, Narod SA, Xie Y (2013) Promoter methylation of BRCA1 in triple-negative breast cancer predicts sensitivity to adjuvant chemotherapy. Ann Oncol 24(6):1498–1505. doi:10.1093/annonc/mdt011
28. Sharma G, Mirza S, Parshad R, Srivastava A, Gupta SD, Pandya P, Ralhan R (2010) Clinical significance of promoter hypermethylation of DNA repair genes in tumor and serum DNA in invasive ductal breast carcinoma patients. Life Sci 87(3–4):83–91. doi:10.1016/j.lfs.2010.05.001
29. Sharma G, Mirza S, Yang YH, Parshad R, Hazrah P, Datta Gupta S, Ralhan R (2009) Prognostic relevance of promoter hypermethylation of multiple genes in breast cancer patients. Cell Oncol 31(6):487–500. doi:10.3233/CLO-2009-0507
30. Karray-Chouayekh S, Trifa F, Khabir A, Boujelbane N, Sellami-Boudawara T, Daoud J, Frikha M, Gargouri A, Mokdad-Gargouri R (2009) Clinical significance of epigenetic inactivation of hMLH1 and BRCA1 in Tunisian patients with invasive breast carcinoma. J Biomed Biotechnol 2009:369129. doi:10.1155/2009/369129
31. Jing F, Jun L, Yong Z, Wang Y, Fei X, Zhang J, Hu L (2008) Multigene methylation in serum of sporadic Chinese female breast cancer patients as a prognostic biomarker. Oncology 75(1–2):60–66. doi:10.1159/000155145
32. Berry DA, Cronin KA, Plevritis SK, Fryback DG, Clarke L, Zelen M, Mandelblatt JS, Yakovlev AY, Habbema JD, Feuer EJ (2005) Effect of screening and adjuvant therapy on mortality from breast cancer. N Engl J Med 353(17):1784–1792. doi:10.1056/NEJMoa050518
33. Hayes DF, Isaacs C, Stearns V (2001) Prognostic factors in breast cancer: current and new predictors of metastasis. J Mammary Gland Biol Neoplasia 6(4):375–392
34. Ronneberg JA, Fleischer T, Solvang HK, Nordgard SH, Edvardsen H, Potapenko I, Nebdal D, Daviaud C, Gut I, Bukholm I, Naume B, Borresen-Dale AL, Tost J, Kristensen V (2011) Methylation profiling with a panel of cancer related genes: association with estrogen receptor, TP53 mutation status and expression subtypes in sporadic breast cancer. Mol Oncol 5(1):61–76. doi:10.1016/j.molonc.2010.11.004
35. Kennedy RD, Quinn JE, Johnston PG, Harkin DP (2002) BRCA1: mechanisms of inactivation and implications for management of patients. Lancet 360(9338):1007–1014. doi:10.1016/S0140-6736(02)11087-7
36. Stefansson OA, Villanueva A, Vidal A, Marti L, Esteller M (2012) BRCA1 epigenetic inactivation predicts sensitivity to platinum-based chemotherapy in breast and ovarian cancer. Epigenetics 7(11):1225–1229. doi:10.4161/epi.22561
37. Krasteva ME, Bozhanov SS, Antov GG, Gospodinova ZI, Angelov SG (2012) Breast cancer patients with hypermethylation in the promoter of BRCA1 gene exhibit favorable clinical status. Neoplasma 59(1):85–91
38. Jiang Y, Cui L, Chen WD, Shen SH, Ding LD (2012) The prognostic role of RASSF1A promoter methylation in breast cancer: a meta-analysis of published data. PLoS One 7(5):e36780. doi:10.1371/journal.pone.0036780
39. Hamilton MG, Roldan G, Magliocco A, McIntyre JB, Parney I, Easaw JC (2011) Determination of the methylation status of MGMT in different regions within glioblastoma multiforme. J Neurooncol 102(2):255–260. doi:10.1007/s11060-010-0307-5
40. Wu TL, Zhang D, Chia JH, Tsao K, Sun CF, Wu JT (2002) Cell-free DNA: measurement in various carcinomas and establishment of normal reference range. Clin Chim Acta 321(1–2):77–87
41. Kohler C, Barekati Z, Radpour R, Zhong XY (2011) Cell-free DNA in the circulation as a potential cancer biomarker. Anticancer Res 31(8):2623–2628
42. Ignatiadis M, Reinholz M (2011) Minimal residual disease and circulating tumor cells in breast cancer. Breast Cancer Res 13(5):222. doi:10.1186/bcr2906
43. Dumitrescu RG (2012) Epigenetic markers of early tumor development. Methods Mol Biol 863:3–14. doi:10.1007/978-1-61779-612-8_1
44. Wooster R, Neuhausen SL, Mangion J, Quirk Y, Ford D, Collins N, Nguyen K, Seal S, Tran T, Averill D et al (1994) Localization of a breast cancer susceptibility gene, BRCA2, to chromosome 13q12-13. Science 265(5181):2088–2090
45. Ben Gacem R, Hachana M, Ziadi S, Amara K, Ksia F, Mokni M, Trimeche M (2012) Contribution of epigenetic alteration of BRCA1 and BRCA2 genes in breast carcinomas in Tunisian patients. Cancer Epidemiol 36(2):190–197. doi:10.1016/j.canep.2011.09.001
46. Nasir A, Shackelford RE, Anwar F, Yeatman TJ (2009) Genetic risk of breast cancer. Minerva Endocrinol 34(4):295–309