RESEARCH ARTICLE

Aberrant promoter methylation of *HIN-1* gene may contribute to the pathogenesis of breast cancer: a meta-analysis

Di Dai · Xi-Hua Dong · Shi-Tong Cheng · Ge Zhu · Xiao-Lin Guo

Abstract We conducted the present meta-analysis of relevant cohort studies to evaluate whether promoter methylation of the high in normal-1 (*HIN-1*) gene contributes to breast cancer. The MEDLINE (1966~2013), Cochrane Library (Issue 12, 2013), EMBASE (1980~2013), CINAHL (1982~2013), Web of Science (1945~2013), and Chinese Biomedical (CBM) (1982~2013) databases were searched without any language restrictions. Meta-analyses were conducted using Stata software (version 12.0; Stata Corporation, College Station, TX, USA). Crude odds ratios (ORs) with their 95 % confidence interval (CI) were calculated. Nine clinical cohort studies that enrolled a total of 693 breast cancer patients were included in the meta-analysis. The results of our meta-analysis demonstrated that *HIN-1* methylation frequency in cancer tissue was significantly higher than that of normal and benign tissues (cancer tissue vs. normal tissue: OR=52.60, 95 % CI= 33.77~81.92, $P<0.001$; cancer tissue vs. benign tissue: OR= 2.38, 95 % CI=1.53~3.70, $P<0.001$; respectively). Ethnicity-stratified analysis indicated that *HIN-1* promoter methylation was correlated with the pathogenesis of breast cancer among both Asians and Caucasians (all $P<0.05$). Our findings provide empirical evidence that aberrant *HIN-1* promoter methylation may contribute to the pathogenesis of breast cancer. Thus, aberrant *HIN-1* promoter methylation could be an independent and important biomarker used in predicting the prognosis and progression of breast cancer.

Keywords *HIN-1* · Promoter methylation · Breast cancer · Meta-analysis

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Introduction

Breast cancer, which occurs in breast tissue, is the most frequently diagnosed malignancy and the leading cause of cancer-related death among women; it is responsible for about 458,400 deaths in 2008 worldwide, accounting for 14 % of the total cancer deaths [1]. According to statistics, an estimated 234,580 new breast cancer cases were diagnosed, accounting for 29 % of all new malignancies among women, and approximately 40,030 patients died of breast cancer in the USA in 2013 [2]. In addition, it has been reported that the incidence of breast cancer varies greatly across regions and countries, with the highest in more developed countries and the lowest in less developed countries [2, 3]. As a complex multifactorial disease, breast cancer has been widely accepted to be induced by interactions between environmental and genetic factors [4, 5]. Numerous studies have showed that various factors such as age, cigarette smoking, alcohol consumption, unhealthy lifestyle, obesity, breastfeeding, and physical activity may be significantly related with an increased risk of breast cancer [6, 7]. Despite these environmental factors, increasing evidence has demonstrated that promoter cytosine-guanine (CpG) islands hypermethylation of tumor-related genes may contribute to the pathogenesis of breast cancer in recent years [8, 9].

High in normal-1 (HIN-1), also known as secretoglobin 3A1 or uteroglobin-related protein 2, is a member of secretoglobin family that consists of small secretory proteins [10]. HIN-1 expression relates to terminally differentiated bronchial epithelial cells, suggesting that HIN-1 is expressed in many epithelial tissues and may be involved in regulating epithelial cell proliferation, differentiation, and morphogenesis [11]. Thus, loss of HIN-1 expression may result in the development and progress of the malignant phenotype in human tumors [12]. Actually, HIN-1 expression is downregulated in the majority of solid tumors, including those of the

lung, pancreas, and breast, prostate, and salivary gland [13]. The human *HIN-1* gene is located at chromosome 5q35.3 and encodes a protein of 104 amino acids, which contains a signal peptide sequence of 20 amino acid residues [14]. HIN-1 was discovered by serial analysis of gene expression (SAGE) and has been found to be highly expressed in normal breast tissues but silenced by methylation in the majority of cancerous breast tissues as well as in preinvasive lesions [14, 15]. It is well accepted that aberrant methylation of the *HIN-1* gene often occurs in multiple human cancer types at an early stage of malignant transformation, indicating that *HIN-1* gene is a candidate tumor suppressor gene [12]. Specifically, methylation of the *HIN-1* gene may have an effect on normal cell cycle regulation, DNA repair, and apoptosis and may thereby cause the transcriptional silencing and result in the development of many malignancies [16]. In this regard, it is plausible to postulate that aberrant methylation of the *HIN-1* gene associated with gene inactivation may be an early event in the pathogenesis of breast cancer. In resent research, methylation patterns of the *HIN-1* gene promoter have been extensively studied, and a close correlation between *HIN-1* gene methylation and malignant phenotypes has been well established in breast cancer [17, 18]. However, contradictory results have also been reported [19, 20]. Given the conflicting evidence on this issue, we performed a meta-analysis of all available cohort studies to determine the relationships between aberrant promoter methylation of *HIN-1* gene and the pathogenesis of breast cancer.

Materials and methods

Literature search and selection criteria

The Web of Science (1945~2013), Cochrane Library (Issue 12, 2013), PubMed (1966~2013), EMBASE (1980~2013), CINAHL (1982~2013), and Chinese Biomedical (CBM) (1982~2013) databases were searched without language restrictions. We used the following keywords and MeSH terms in conjunction with a highly sensitive search strategy: ("methylation" or "hypermethylation" or "DNA methylation" or "demethylation" or "promoter methylation") and ("SCGB3A1 protein, human" or "SCGB3A1" or "UGRP2" or "uteroglobin-related protein 2" or "HIN-1" or "high in normal-1") and ("breast neoplasms" or "breast cancer" or "breast tumor" or "breast carcinoma" or "mammary cancer" or "mammary carcinoma" or "mammary neoplasms" or "mastocarcinoma"). A manual search on the basis of references identified in the included articles was carried out to obtain other potential articles.

Fig. 1 Flow chart shows study selection procedure. Nine cohort studies were included in this meta-analysis
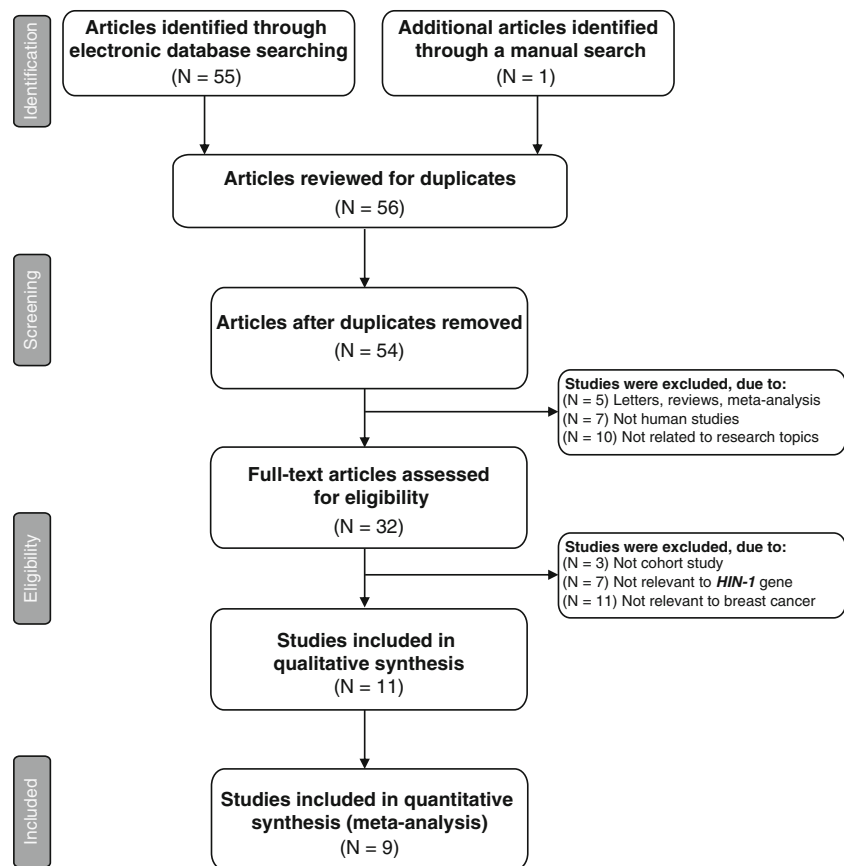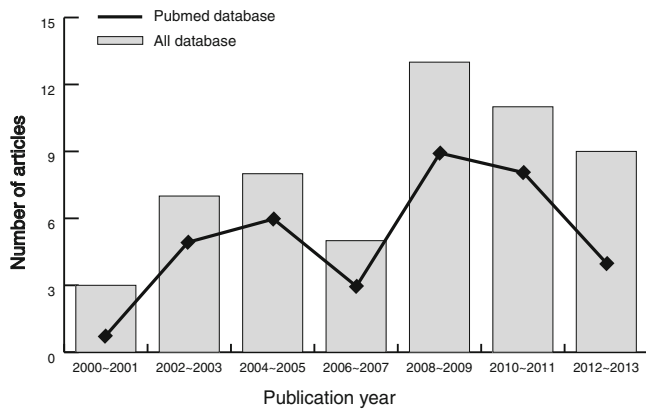
Fig. 2 Distribution of the number of topic-related literature in electronic databases over the last decade

The following criteria were used to determine the eligibility of included studies: (1) study concerns the correlations between *HIN-1* promoter methylation and breast cancer, (2) all patients met the diagnostic criteria of breast cancer and were confirmed by histopathologic examinations, and (3) study provides sufficient information about the frequency of *HIN-1* gene methylation. Articles that did not meet these inclusion criteria were excluded. If several studies were conducted using the same subjects, either the most recent or largest sample size publication was included.

Data extraction and methodological assessment

Two authors used a standardized form to extract the following data from included studies: language of publication, publication year of article, the first author's surname, geographical location, design of study, total number of cases, sample size, the source of controls, detection method of methylation, and the frequency of methylation.

Two researchers independently assessed methodological quality using the Newcastle-Ottawa Scale (NOS) criteria [21]. The NOS criteria is comprised of three aspects: (1) subject selection, 0~4; (2) comparability of subject, 0~2;

and (3) clinical outcome, 0~3. Total NOS scores range from 0 to 9 with a score of ≥7 indicating good quality.

Statistical analysis

All analyses were conducted using Stata statistical software (version 12.0; Stata Corporation, College Station, TX, USA). Odds ratios (ORs) and their corresponding 95 % confidence intervals (CIs) were calculated. The statistical significance of pooled ORs was evaluated by using the Z test. Between-study heterogeneity was assessed by Cochran's Q statistic and I^2 tests [22]. When a P value of <0.05 or I^2>50 % indicated that these studies were heterogeneous, the random effects model was conducted. Otherwise, the fixed effects model was implemented. We also conducted subgroup analyses to explore potential sources of the heterogeneity. In order to evaluate the influence of single studies on the overall estimate, a sensitivity analysis was employed. Potential publication bias was examined by funnel plots and Egger's linear regression test [23].

Results

Baseline characteristics of included studies

A total of 56 articles relevant to the searched keywords were initially identified. After reviewing the titles and abstracts of all articles, we excluded 22 articles. Full texts and data integrity were then reviewed, and another 22 articles were excluded. Another two studies were excluded due to a lack of data integrity (Fig. 1). Eventually, nine clinical cohort studies with a total of 693 breast cancer patients met our inclusion criteria for quantitative data analysis [14, 17–20, 24–27]. The range of publication years of the eligible studies was from 2001 to 2013. The distribution of the number of topic-related literatures in the electronic database during the last decades is depicted in Fig. 2. Overall, three studies were performed

Table 1 Main characteristics and methodological quality of all eligible studies

| First author	Year	Ethnicity	Sample size	Age (years)	Detection method	NOS score
Twelves [19]	2013	Caucasians	54	52	MSP	6
Verschuur-Maes [18]	2012	Caucasians	56	–	MS-MLPA	5
Park [20]	2011	Asians	85	50.7±11.2	MethyLight assay	7
Kim [26]	2010	Asians	119	51 (25~82)	QM-MSP	8
Feng [11]	2007	Asians	38	59 (35~84)	Bisulfite pyrosequencing	7
Lee [17]	2008	Asians/Caucasians	48/52	47.0±10.0/51.0±12.0	QM-MSP	7
Shigematsu [27]	2005	Caucasians	37	–	MSP	6
Fackler [24]	2003	Caucasians	204	51 (43~66)	MSP	7

NOS Newcastle-Ottawa Scale criteria, *MSP* methylation-specific, *PCR* polymerase chain reaction, *MS-MLPA* methylation-specific multiplex ligation-dependent probe amplification, *QM-MSP* quantitative multiplex MSP

among Asians, five studies among Caucasians, and the other one study among mixed populations. Methylation-specific PCR (MSP), methylation-specific multiplex ligation-dependent probe amplification (MS-MLPA), MethyLight assay, quantitative multiplex MSP (QM-MSP), and bisulfite pyrosequencing were employed to investigate the methylation status of *HIN-1* gene. We summarized the study characteristics and methodological qualities in Table 1.

Quantitative data synthesis

Our results demonstrated that the frequency of *HIN-1* gene methylation in cancer tissue was significantly higher than that in normal and benign tissues (cancer tissue vs. normal tissue: OR=52.60, 95 % CI=33.77~81.92, *P*<0.001; cancer tissue vs. benign tissue: OR=2.38, 95 % CI=1.53~3.70, *P*<0.001; respectively) (Fig. 3).

Fig. 3 Forest plot for the associations between aberrant *HIN-1* promoter methylation and the pathogenesis of breast cancer
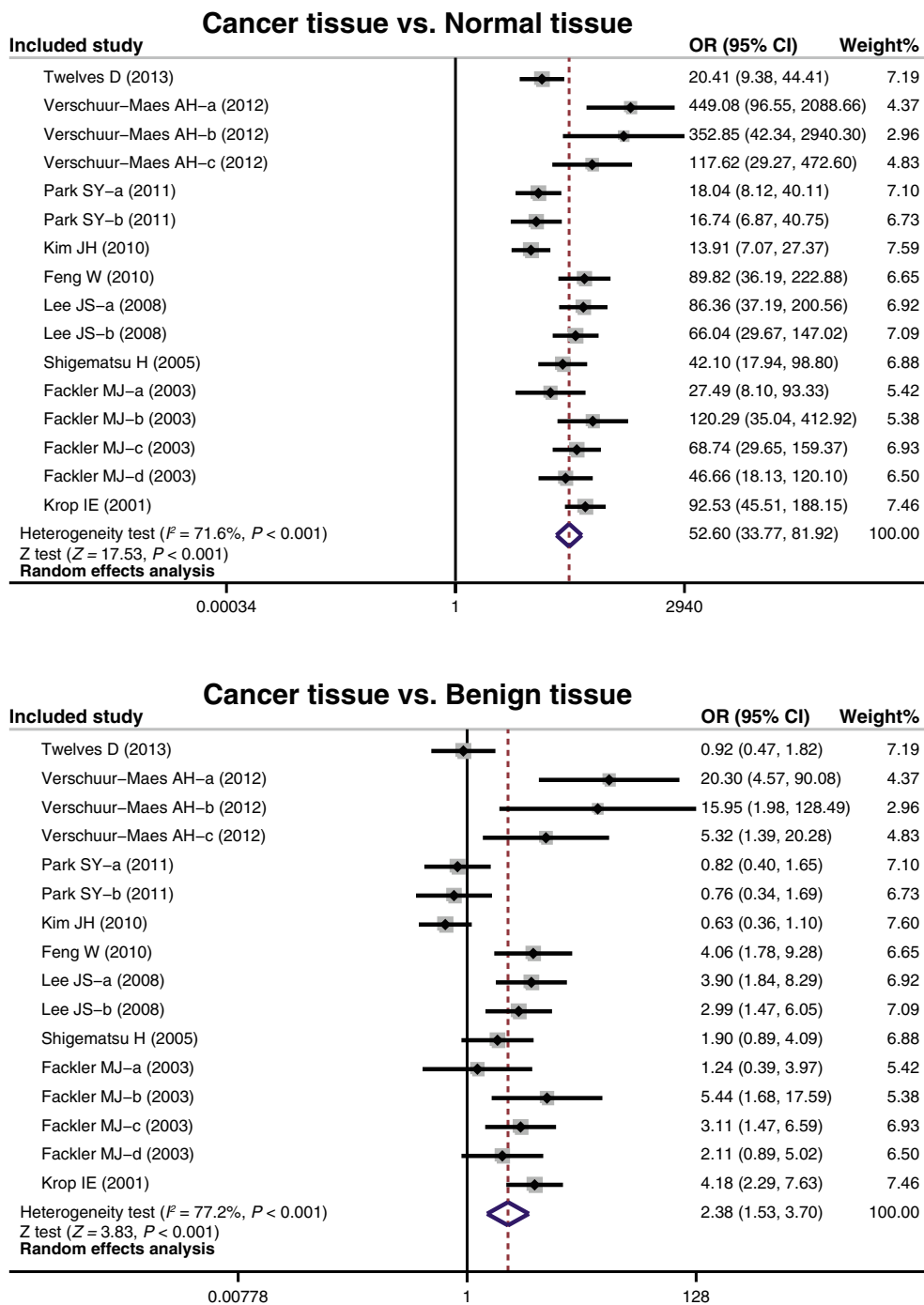
To comprehensively evaluate the effects of *HIN-1* promoter methylation on the pathogenesis of breast cancer, we also carried out subgroup analyses based on ethnicity and detection method. Ethnicity-stratified analysis indicated that there were significantly higher frequencies of *HIN-1* promoter methylation in cancer tissue than in normal tissues among both Caucasians and Asians (Caucasians: OR=67.36, 95 % CI= 42.26~107.37, *P*<0.001; Asians: OR=31.19, 95 % CI= 13.86~70.18, *P*<0.001; respectively) (Fig. 4). Furthermore, we found that the frequency of aberrant *HIN-1* gene methylation in cancer tissue was higher than that in benign tissue among Caucasians and Asians (OR=3.03, 95 % CI=1.92~ 4.80, *P*<0.001; Asians: OR=1.41, 95 % CI=1.03~3.86, *P*= 0.045; respectively) (Fig. 4). As for the subgroup analysis based on detection method, our findings reveal that the aberrant methylation of *HIN-1* was correlated with the pathogenesis of breast cancer in the majority of subgroups (Fig. 4).

A sensitivity analysis was carried out to assess the effects of each individual study on the pooled estimates by sequentially excluding individual studies. The results indicate that the overall pooled estimates could not be influenced by any single study (Fig. 5). Funnel plots showed an obvious asymmetry (Fig. 6), and Egger's test also presented strong evidence of publication bias (cancer tissue vs. normal tissue: *t*=2.40, *P*= 0.031; cancer tissue vs. benign tissue: *t*=2.27, *P*=0.039; respectively).

Discussion

The results of our meta-analysis reveal that the frequency of *HIN-1* promoter methylation in cancer tissue was significantly higher than that in normal and benign tissues, suggesting that the aberrant methylation of the *HIN-1* gene may be implicated
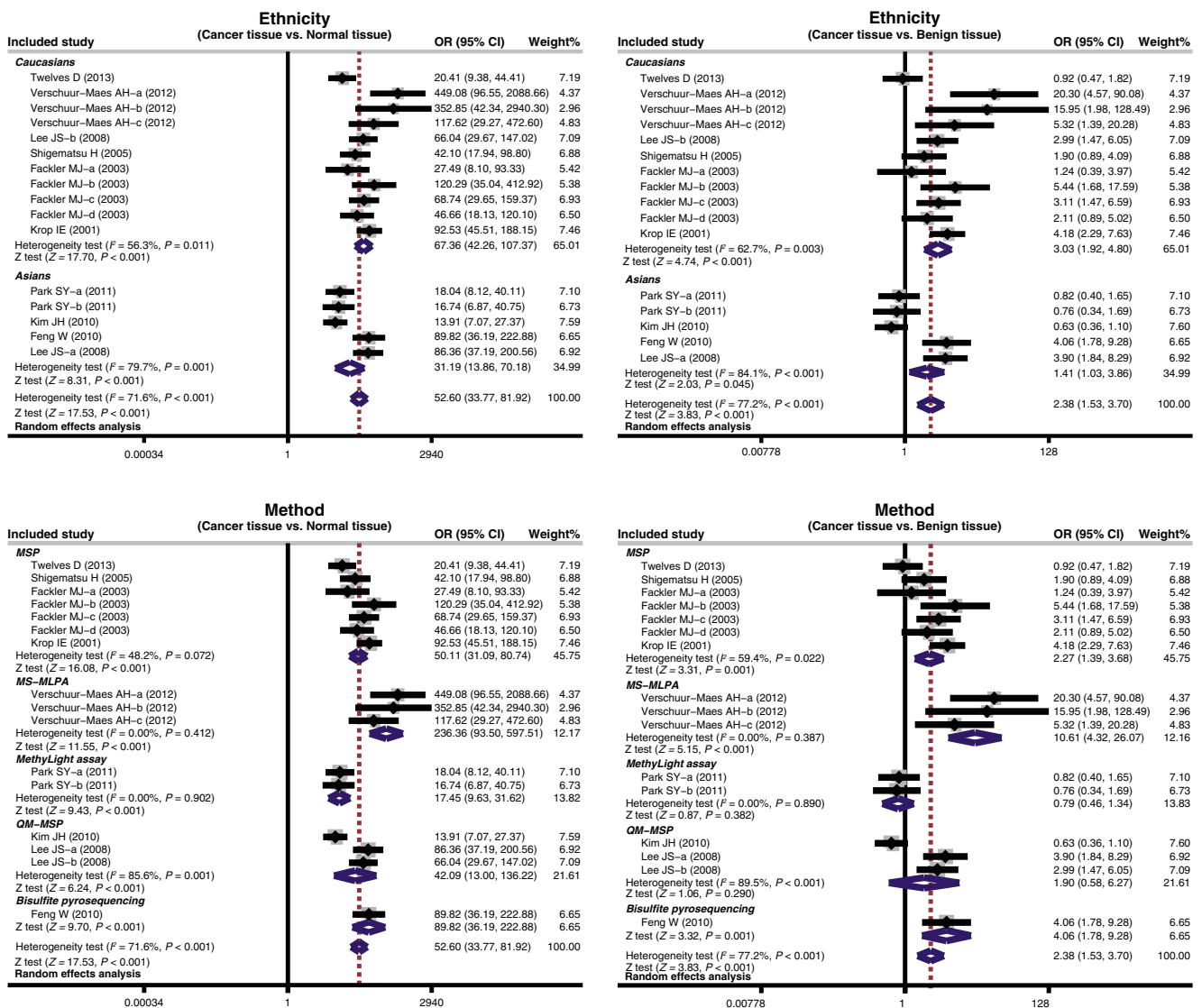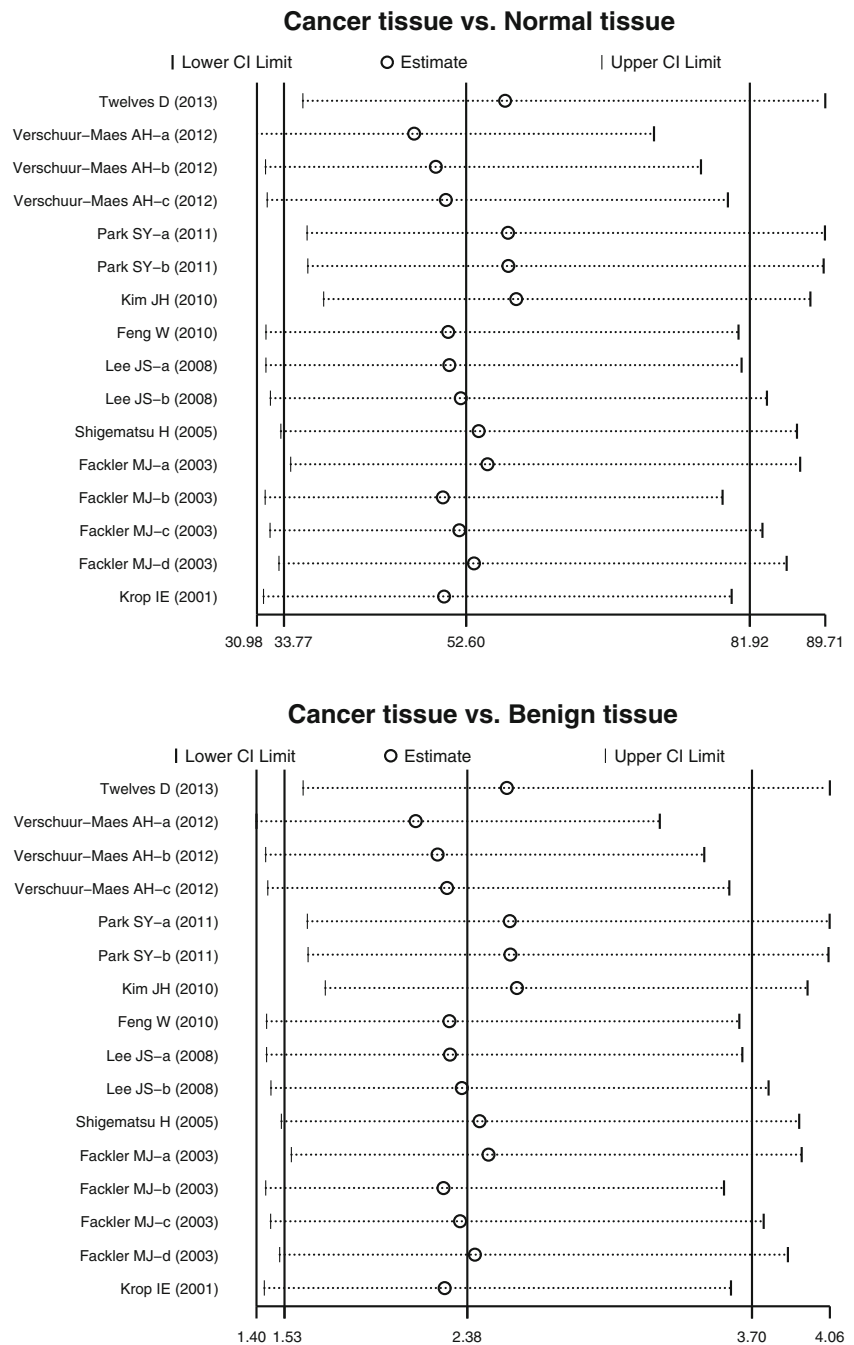
Fig. 4 Subgroup analyses of the relationships between aberrant *HIN-1* promoter methylation and the pathogenesis of breast cancer

Fig. 5 Sensitivity analysis of the summary odds ratio coefficients on the associations between aberrant *HIN-1* promoter methylation and the pathogenesis of breast cancer

in the pathogenesis of breast cancer. It has been previously considered that *HIN-1* is a potential tumor suppressor based on studies demonstrating that the overexpression of HIN-1 is capable of inhibiting growth in several human cancer cell lines and is usually downregulated in invasion and in situ carcinomas by promoter hypermethylation [24]. Nevertheless, the precise mechanisms by which the *HIN-1* aberrant methylation leads to an increased risk of breast cancer are still poorly understood. It is noteworthy that HIN-1 is a putative cytokine to be highly expressed in the normal terminal duct lobular unit (TDLU) and may be involved in regulating epithelial cell proliferation, differentiation, and morphogenesis. Additionally, the downregulation of HIN-1 by promoter hypermethylation was observed in a high percentage of primary breast tumors [23]. Furthermore, hypermethylation of the CpG island of genes is associated with transcriptional gene silencing in all types of human cancers and may possibly contribute to uncontrolled tumor cells growth [28]. In this regard, the higher prevalence of *HIN-1* promoter methylation may lead to the silencing of HIN-1 expression and result in the unlimited growth of tumor cells, promoting primary breast cancers to lymph node metastases during tumor progression
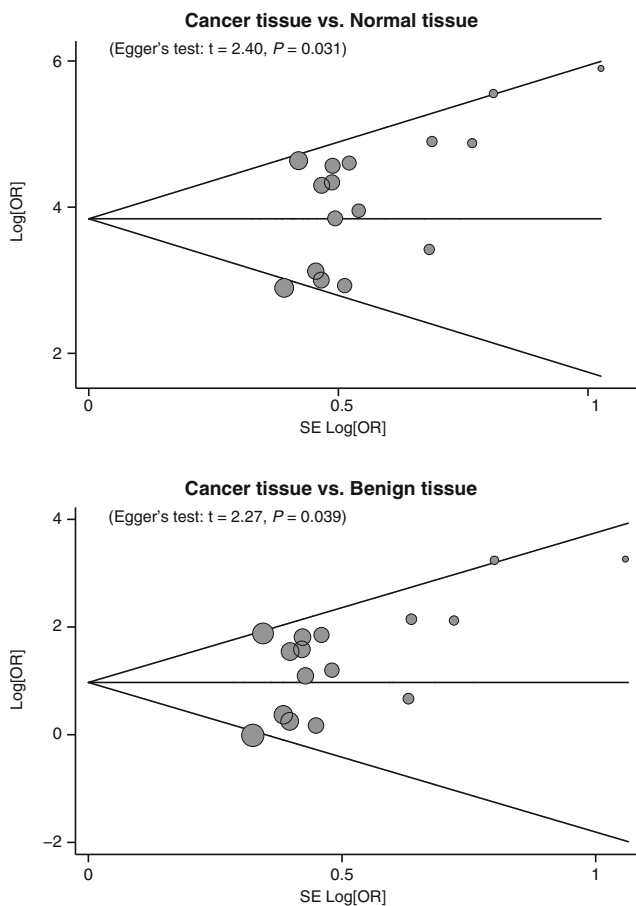
Fig. 6 Funnel plot of publication biases on the associations between aberrant *HIN-1* promoter methylation and the pathogenesis of breast cancer

[25]. On the other hand, it can be postulated that the inhibition of the DNA methylation can activate silenced tumor suppressor genes to some extent, which is associated with the arrest of tumor growth. Therefore, aberrant methylation of *HIN-1*, as an early event in carcinogenesis, could serve as a potential biomarker for the early diagnosis of breast cancer. In accordance with our results, Park et al. also concluded that the CpG island methylation of tumor-related *HIN-1* gene is an early event that predominantly occurs during the preinvasive stage of BC and accumulates with breast cancer progression [20]. Furthermore, Shigematsu et al. also determined that *HIN-1* promoter methylation was a common and important epigenetic mechanism for the inactivation of HIN-1 function and may be a new potential biomarker that could predicted the biological and clinical status of breast cancer [27]. Given the presence of heterogeneity between studies, a stratified analysis was carefully performed according to ethnicity. Our results showed that the aberrant methylation of the *HIN-1* gene was significantly related to the pathogenesis of breast cancer among both Caucasians and Asians.

Our study has several potential limitations that should be considered when interpreting our results. First, due to the small number of included studies, our results may not have included all data from all trials that have evaluated the relationship of *HIN-1* promoter methylation with susceptibility to breast cancer. Nevertheless, our meta-analysis managed to overcome the size and scope limitations of individual studies by systematically aggregating results of each study in order to acquire a more reliable and comprehensive estimation of the correlation between *HIN-1* promoter methylation and the pathogenesis of breast cancer. The second limitation is the fact that obvious statistical heterogeneity existed between the included studies, which may have been caused by a number of factors such as differences in subject ethnicity, study design, and genotyping method. In addition, our meta-analysis was unable to acquire the original data from the included studies. Despite the above limitations, this is the first meta-analysis on the association between *HIN-1* promoter methylation and the pathogenesis of breast cancer. In addition, our meta-analysis employed a comprehensive literature search strategy, strict inclusion and exclusion criteria, as well as rigorous statistical analyses, which ensured the robustness and reliability of our results.

In brief, our findings provide empirical evidence that aberrant *HIN-1* promoter methylation may contribute to the pathogenesis of breast cancer. Thus, aberrant *HIN-1* promoter methylation could be an independent and important biomarker used in predicting the prognosis and progression of breast cancer. However, given the limitations of this meta-analysis, further large sample size studies are needed to achieve a more reliable and generally applicable statistical analysis.

Conflicts of interest None.

References

1. Jemal A, Bray F, Center MM, Ferlay J, Ward E, Forman D. Global cancer statistics. CA Cancer J Clin. 2011;61(2):69–90.
2. Siegel R, Naishadham D, Jemal A. Cancer statistics, 2013. CA Cancer J Clin. 2013;63(1):11–30.
3. Forouzanfar MH, Foreman KJ, Delossantos AM, Lozano R, Lopez AD, Murray CJ, et al. Breast and cervical cancer in 187 countries between 1980 and 2010: a systematic analysis. Lancet. 2011;378(9801): 1461–84.
4. Nelson HD, Zakher B, Cantor A, Fu R, Griffin J, O'Meara ES, et al. Risk factors for breast cancer for women aged 40 to 49 years: a systematic review and meta-analysis. Ann Intern Med. 2012;156(9):635–48.
5. Campa D, Kaaks R, Le Marchand L, Haiman CA, Travis RC, Berg CD, et al. Interactions between genetic variants and breast cancer risk factors in the breast and prostate cancer cohort consortium. J Natl Cancer Inst. 2011;103(16):1252–63.

6. Barnett GC, Shah M, Redman K, Easton DF, Ponder BA, Pharoah PD. Risk factors for the incidence of breast cancer: do they affect survival from the disease? J Clin Oncol. 2008;26(20):3310–6.

7. Park SK, Kim Y, Kang D, Jung EJ, Yoo KY. Risk factors and control strategies for the rapidly rising rate of breast cancer in Korea. J Breast Cancer. 2011;14(2):79–87.

8. Hill VK, Ricketts C, Bieche I, Vacher S, Gentle D, Lewis C, et al. Genome-wide DNA methylation profiling of CpG islands in breast cancer identifies novel genes associated with tumorigenicity. Cancer Res. 2011;71(8):2988–99.

9. Sun Z, Asmann YW, Kalari KR, Bot B, Eckel-Passow JE, Baker TR, et al. Integrated analysis of gene expression, CpG island methylation, and gene copy number in breast cancer cells by deep sequencing. PLoS ONE. 2011;6(2):e17490.

10. Tomita T, Kimura S. Regulation of mouse Scgb3a1 gene expression by NF-Y and association of CpG methylation with its tissue-specific expression. BMC Mol Biol. 2008;9:5.

11. Feng W, Shen L, Wen S, Rosen DG, Jelinek J, Hu X, et al. Correlation between CpG methylation profiles and hormone receptor status in breast cancers. Breast Cancer Res. 2007;9(4):R57.

12. Ahlquist T, Lind GE, Costa VL, Meling GI, Vatn M, Hoff GS, et al. Gene methylation profiles of normal mucosa, and benign and malignant colorectal tumors identify early onset markers. Mol Cancer. 2008;7:94.

13. Wu Q, Lothe RA, Ahlquist T, Silins I, Trope CG, Micci F, et al. DNA methylation profiling of ovarian carcinomas and their in vitro models identifies HOXA9, HOXB5, SCGB3A1, and CRABP1 as novel targets. Mol Cancer. 2007;6:45.

14. Krop IE, Sgroi D, Porter DA, Lunetta KL, LeVangie R, Seth P, et al. HIN-1, a putative cytokine highly expressed in normal but not cancerous mammary epithelial cells. Proc Natl Acad Sci U S A. 2001;98(17):9796–801.

15. Guo M, Ren J, Brock MV, Herman JG, Carraway HE. Promoter methylation of HIN-1 in the progression to esophageal squamous cancer. Epigenetics. 2008;3(6):336–41.

16. Gong Y, Guo MZ, Ye ZJ, Zhang XL, Zhao YL, Yang YS. Silence of HIN-1 expression through methylation of its gene promoter in gastric cancer. World J Gastroenterol. 2011;17(4):526–33.

17. Lee JS, Fackler MJ, Teo WW, Lee JH, Choi C, Park MH, et al. Quantitative promoter hypermethylation profiles of ductal carcinoma in situ in North American and Korean women:

18. Verschuur-Maes AH, de Bruin PC, van Diest PJ. Epigenetic progression of columnar cell lesions of the breast to invasive breast cancer. Breast Cancer Res Treat. 2012;136(3):705–15.

19. Twelves D, Nerurkar A, Osin P, Dexter T, Ward A, Gui GP, et al. DNA promoter hypermethylation profiles in breast duct fluid. Breast Cancer Res Treat. 2013;139(2):341–50.

20. Park SY, Kwon HJ, Lee HE, Ryu HS, Kim SW, Kim JH, et al. Promoter CpG island hypermethylation during breast cancer progression. Virchows Arch. 2011;458(1):73–84.

21. Stang A. Critical evaluation of the Newcastle-Ottawa scale for the assessment of the quality of nonrandomized studies in meta-analyses. Eur J Epidemiol. 2010;25(9):603–5.

22. Zintzaras E, Ioannidis JP. HEGESMA: genome search meta-analysis and heterogeneity testing. Bioinformatics. 2005;21(18):3672–3.

23. Peters JL, Sutton AJ, Jones DR, Abrams KR, Rushton L. Comparison of two methods to detect publication bias in meta-analysis. JAMA. 2006;295(6):676–80.

24. Fackler MJ, McVeigh M, Evron E, Garrett E, Mehrotra J, Polyak K, et al. DNA methylation of RASSF1A, HIN-1, RAR-beta, Cyclin D2 and Twist in in situ and invasive lobular breast carcinoma. Int J Cancer. 2003;107(6):970–5.

25. Feng W, Orlandi R, Zhao N, Carcangiu ML, Tagliabue E, Xu J, et al. Tumor suppressor genes are frequently methylated in lymph node metastases of breast cancers. BMC Cancer. 2010;10:378.

26. Kim JH, Shin MH, Kweon SS, Park MH, Yoon JH, Lee JS, et al. Evaluation of promoter hypermethylation detection in serum as a diagnostic tool for breast carcinoma in Korean women. Gynecol Oncol. 2010;118(2):176–81.

27. Shigematsu H, Suzuki M, Takahashi T, Miyajima K, Toyooka S, Shivapurkar N, et al. Aberrant methylation of HIN-1 (high in normal-1) is a frequent event in many human malignancies. Int J Cancer. 2005;113(4):600–4.

28. Buchmann RC, Asad S, Wolf JN, Mohannath G, Bisaro DM. Geminivirus AL2 and L2 proteins suppress transcriptional gene silencing and cause genome-wide reductions in cytosine methylation. J Virol. 2009;83(10):5005–13.