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Association of Methylation of RASSF1A between Breast Cancer and Benign Breast Lesions: A Meta-Analysis

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Abstract

Objective: To systematically evaluate the relationship between methylation of RASSF1A and breast cancer. And to evaluate the association of methylation of RASSF1A between breast cancer and benign breast lesions.

Methods: Screen the literatures that retrieved from PubMed, Cochrane Library and Ovid. Evaluate the quality of included studies. Data analysis performed by RevMan5.3 and Stata12.0 software.

Results: A total of 7 articles were included in this meta-analysis, including 1,485 samples. The results indicate that the positive rate of RASSF1A methylation have conspicuous difference between breast cancer and normal group, between breast cancer and benign breast lesion, as well. Respectively (BC *vs.* N, OR=4.52, 95% CI (2.02~10.10), P=0.0002; BC *vs.* BBL, OR=3.63, 95% CI (2.50~5.43), P=0.0002; BBL *vs.* N, OR=0.83, 95% CI (0.53~1.30), P=0.42).

Conclusion: This meta-analysis confirms that hyper-methylation of RASSF1A is closely related to breast tumor. And it is of significance in differentiating benign breast lesions from malignant breast lesions.

Keywords: RASSF1A; Methylation; Breast cancer; Benign breast lesions; Meta-Analysis

Abbreviations

RASSF1A: RAS Association Domain Family 1A; BC: Breast Cancer; N: Normal; BBL: Benign Breast Lesion

Introduction

Breast cancer is the most common malignancy in women and is one of the three most frequently diagnosed cancers relating death in the world [1]. Every year, approximately 1.7 million women are diagnosed with breast cancer and about 0.5 million die [2]. In recent years, a lot of scholars have paid close attention to the molecular mechanism of breast cancer.

Genetic and epigenetic changes are closely related to malignant tumors, and have become a research hotspot in the diagnosis of breast cancer. Methylation of tumor suppressor genes, known as epigenetic alterations, have turned out to be a frequent event in development, differentiation and tissue homeostasis [3,4] of breast tumor. Methylation of particular genes is one of the earliest detectable changes in a tumor, and in some cases may occur even before the tumor is formed [5].

Loss of genetic material on 3p21.3 chromosome is one of the most frequent and earliest events in the pathogenesis of many tumors [6]. The RASSF1A gene, RAS-association domain family 1 isoform A, which located in chromosome 3p21.3, is composed of 8 exons and produces 7 transcripts [5-7]. This gene encodes a protein which is a member of RAS effectors. Assessment of Hypermethylation. Initial studies suggested that RASSF1A would be a suppressor gene for breast cancers [8]. Many studies have represented that RASSF1A methylation has remarkable effect on the biological characteristics of breast cancer. However, the methylation analysis techniques used in each study are different, as well as the CpG sites that studied, therefore, the methylation level of RASSF1A may different [9]. All of these may have impact on the result of the correlation between RASSF1A methylation level and breast cancer, to some extent. To assess the association between RASSF1A methylation and breast cancer, as well as the difference between benign and malignant breast lesions, this meta-analysis and systematic review is performed.

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Materials and Methods

Literature searching

Using "breast cancer" including the synonyms likes "breast carcinoma", “breast neoplasm", "breast tumor", "breast tumour", "mammary cancer", and "RASSF1A" or "RAS association domain family 1A” to establish a retrieval model for integrated search in foreign databases, such as PubMed, Ovid and Cochrane Library. Furthermore, we scanned the references and applied the related keywords and synonyms to search for additional publication. There were no restrictions on the design and publication status of the studies to make it more sensitive. Relevant studies on methylation of RASSF1A in breast cancer were searched by computer, from the establishment of the database to January 31, 2019.

The inclusion criteria

1. The source of the case is clear, and the diagnosis of breast carcinoma is definite.

2. Frequency of methylation of RASSF1A in breast cancer, benign breast lesions and normal groups all could be found or calculated.

The exclusion criteria

1. Studies reported repeatedly or cannot find full text.

2. Studies that are letters, reviews, meta-analysis, conference abstracts, annunciates, case reports or animal studies.

3. The research object is inconsistent or the data is incomplete.

Records screening

First of all, we screened the title and abstract of the literatures. The records about "Association between methylation of RASSF1A and breast tumor" would be selected to preliminary included group. Then, the documentations including reviews, meta-analysis, conference abstracts, announcements, case reports and animal experiments were excluded. Simultaneously, researches whose objects are inconsistent or whose data is incomplete were excluded. Remove the repeated records. Finally, the literatures that the frequency of RASSF1A methylation in breast carcinoma, benign breast lesions and normal groups could not to be found or calculated were filtered out after reading the full text. And the studies that could not find full text were removed. In this process of literature screening, two researchers (Xin Ling Deng and Chao Si Qian) screened independently, and in case of disagreement, they reached a consensus after discuss.

Quality assessment

The Newcastle-Ottawa scale (NOS) table [10], which is commonly used for assessing the quality of cohort studies and case-control studies, was applied to assess the quality of these included studies. The evaluation includes three aspects: The selection methods and comparability between the case and control group, and the assessment of exposure. The scores of quality assessment range from 0 to 9, and 0-3, 4-6, 7-9 represent low, medium, high quality, respectively. When two reviewers (Xin Ling Deng and Chao Si Qian) had different opinions on it, the two parties would negotiate or request the assistance of another researcher. In order to ensure the high quality of included literatures, the study got a score <5 was removed.

Data extraction

The following information was extracted from the studies by two investigators (Xin Ling Deng and Chao Si Qian) on their own and crosscheck: The first author’s name, publication year, material and method for methylation analysis. At the meantime, the frequency of methylation of RASSF1A in breast cancer, benign breast lesions and normal groups would be extracted into Excel table.

Statistical methods

As assessing of the included Literatures, we calculated Cochran’s Q-test and Higgin’s I^2 to analysis by RevMan5.3. If P>0.05, I^2<50%, indicates that there is low or no statistical heterogeneity between the studies, then fixed-effects model is applied. Otherwise, random-effects model is adopted (P<0.05, I^2>50%). In addition, we constructed funnel plot to evaluate publication bias intuitively. To assess the degree of asymmetry, we made Begg's test by Stata12.0. If Begg's test P>0.10 and the funnel plot was symmetric, it suggests that no significant publication bias. As to sensitivity analysis, the combined effect size was calculated by using the fixed-effect model and the random-effect model respectively. And compare the consistency of results to reflect the reliability of the meta-analysis.

Result

The basic characteristics of the included studies

A total of 1288 literatures were found from the PubMed, Cochrane Library and Ovid. Finally, 7 studies [11-17] were included in this meta-analysis after screening, with 1485 samples, including 642 breast tumor samples, 367 benign breast lesions and 476 normal. The specific screening process was shown in Figure 1. Basic information including the first author, publication year, samples and methods of methylation of the included articles were extracted in Table 1, and the results of methodological quality evaluation were displayed in Table 2.

Heterogeneity test

Breast cancer *vs.* normal (BC *vs.* N): The result (in Figure 2) reveals that there was moderate statistical heterogeneity between the records (χ^2=0.57, df=18.73 (P=0.005), I^2=68%), therefore, the random-effect model was applied. The combined effect OR=4.52, 95% CI: (2.02~10.10), 95% CI did not include 0, and the P value of Z test (Z=3.67, P=0.0002) was less than 0.1, indicating a statistical difference between the two groups.

Breast cancer *vs.* benign breast lesions (BC *vs.* BBL): The result (in Figure 3) displays that there was low statistical heterogeneity between the articles (χ^2=9.21, df=6 (P=0.16), I^2=35%), therefore, the fixed-effect model was used. The combined effect OR=3.63, 95% CI: (2.50~5.43), 95% CI did not include 0, and the P value of Z test (Z=6.58, P<0.00001) was less than 0.1, indicating a statistical difference between the two groups.

Benign breast lesions *vs.* normal (BBL *vs.* N): The result (in Figure 4) shows that there was no statistical heterogeneity between the studies (χ^2=5.31, df=5 (P=0.38), I^2=6%), therefore, the fixed-

Table 1: Basic information of the included articles.

Author	Year	Country	Sample	Method
Essel Dulaimi	2004	USA	serum	MSP
TE Skvortsova	2006	UK	plasma	MSP
C. Jeronimo	2007	USA	tissue	QMSP
Magdalini Kioulafa	2009	Greece	tissue	MSP
Vera Kloten	2013	Germany	serum	QMSP
Ming Shan	2016	China	serum	Methylight
Y.Ji	2016	China	tissue	MSP

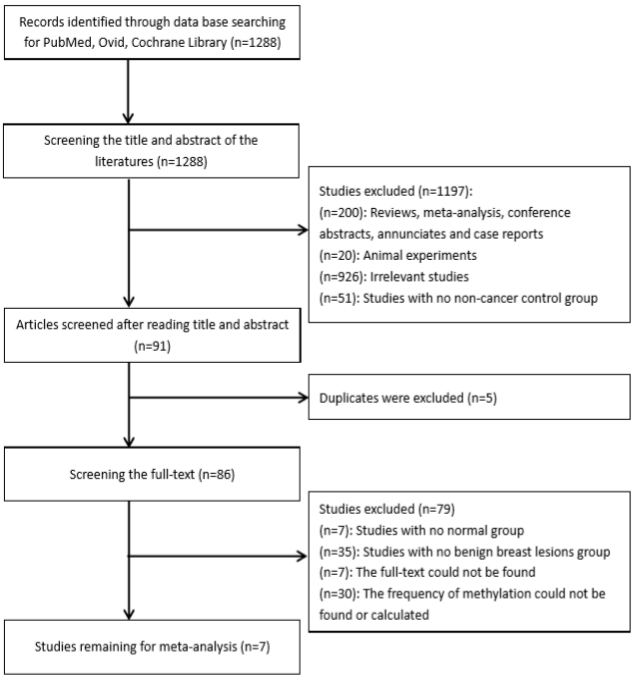

Figure 1: Flow chart of the study selection procedure.

Table 2: Quality assessment of the included articles.

Author	Year	Selection	Comparability	Exposure	NOS
Essel Dulaimi	2004	☆☆☆☆	☆☆	☆☆	8
TE Skvortsova	2006	☆☆☆☆	☆☆	☆☆	8
C. Jeronimo	2007	☆☆☆	☆☆	☆☆	7
Magdalini Kioulafa	2009	☆☆☆	☆☆	☆☆	7
Vera Kloten	2013	☆☆☆☆	☆☆	☆☆	8
Ming Shan	2016	☆☆☆☆	☆☆	☆☆	8
Y. Ji	2016	☆☆☆	☆☆	☆☆	7

effect model was adopted. The combined effect OR=0.83, 95% CI: (0.53~1.30), 95% CI did not include 0, and the P value of Z test (Z=0.81, P=0.42) surpassed 0.1, indicating no statistical difference between the two groups.

Publication bias

There were absence of significant bias in the included studies, which could be seen from the symmetry of the funnel plot in Figure 5-7 and the p-value of Begg's tests (in Table 3) that all exceed 0.1.

Sensitivity analysis

Apply fixed-effect model and random-effect model to calculate the combined effect size respectively. The consistency of results in Table 3 confirms that this meta-analysis was credible.

Table 3: Results of Begg's tests and Sensitivity analysis.

Groups	Begg's test	Fix-effect model	Random-effect model
BC *vs.* N	1	3.15 (2.30~4.31)	4.52 (2.02~10.10)
BC *vs.* BBL	0.548	3.68 (2.50~5.43)	3.93 (2.19~7.06)
BBL *vs.* N	0.707	0.83 (0.53~1.30)	0.78 (0.46~1.29)

Discussion

The RASSF1A, whose official full name is RAS association domain family member 1 A, is located at 3p21.31. The RASSF1A encodes a protein which resembles the RAS effector proteins. The protein can induce cell cycle arrest by inhibiting the cumulation of cyclin D1 [18]. The inactivation of RASSF1A was found to be related to the hyper-methylation of RASSF1A CpG-island promoter region [19,20]. It has been reported that the frequency of RASSF1A methylation in breast cancer patients is higher than in non-cancer patients and healthy controls. However, at present, most studies only compare the cancer group with normal group and only a few studies involve benign groups. The methylation analysis techniques used and the CPG sites studied in each study are not exactly the same. These factors may have some effect on the consistency of the results. In order to get a more accurate result of the correlation between RASSF1A methylation and breast cancer, we performed this meta-analysis with 1,485 cases to determine whether RASSF1A methylation can be used as an indicator in the diagnosis of breast cancer.

Our study showed that the comparison between the breast cancer group and the normal group had statistical significance, and the comparison between the benign lesion group and the malignant lesion group also had statistical significance, while the comparison between the benign lesion group and the normal control group had no statistical significance. It shows that RASSF1A hyper-methylation is positively correlated with the risk of breast cancer and suggests that RASSF1A hyper-methylation has potential value in the diagnosis of breast cancer.

A number of meta-analysis studies have shown that there is a correlation between hyper-methylation of RASSF1A promoter and different types of tumors, such as breast cancer, head and neck squamous cell carcinoma, melanoma, thyroid carcinoma, endometrial cancer, as well as the value of biomarkers for the methylation status of RASSF1A [21-25]. In addition, some studies confirm that RASSF1A methylation could be significant in discriminate cancer from benign lesions [26,27]. Our meta-analysis shows the comparison of methylation of RASSF1A between breast cancer and benign lesions has statistical significance, and the result is consistent with them,

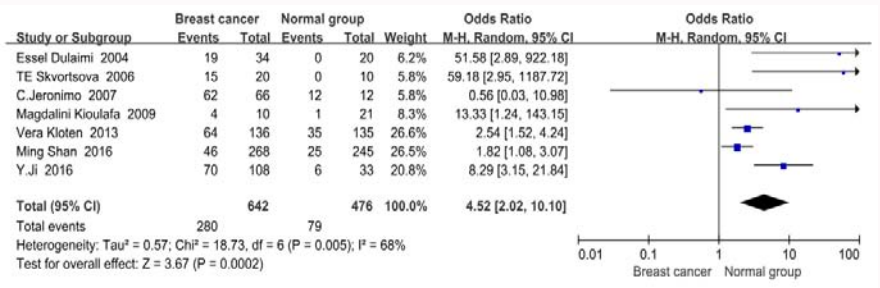

Figure 2: Forest plot of BC *vs.* N.

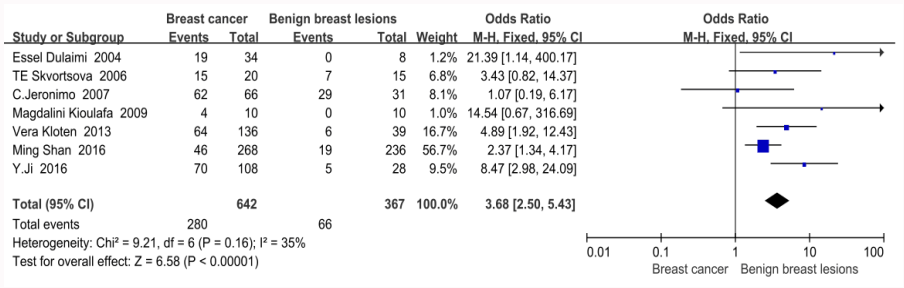

Figure 3: Forest plot of BC *vs.* BBL.

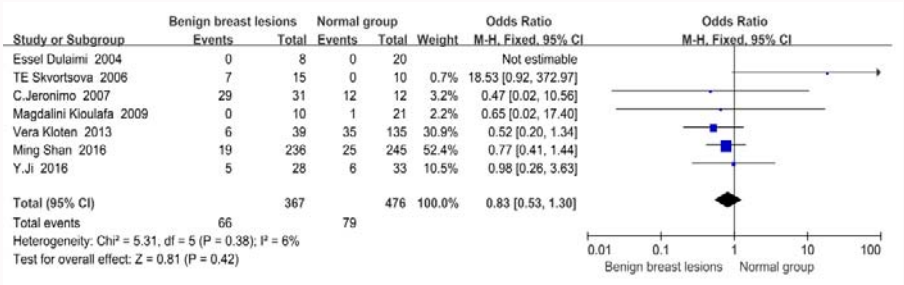

Figure 4: Forest plot of BBL *vs.* N.

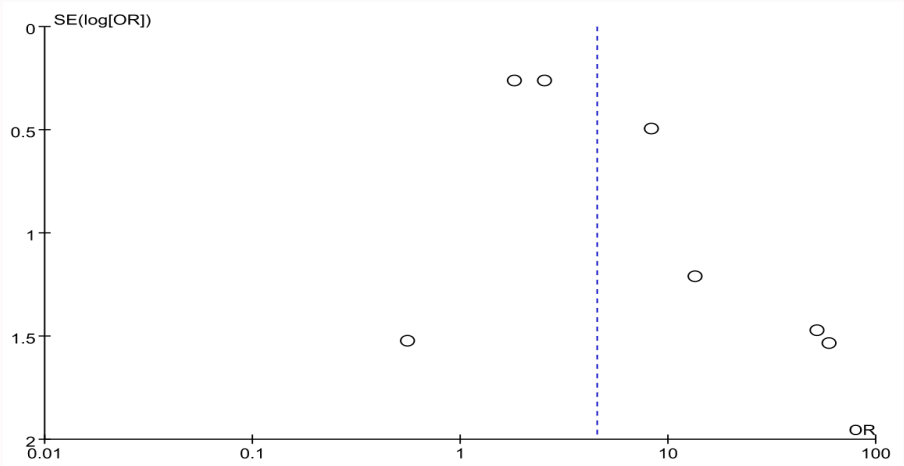

Figure 5: Funnel plot of BC *vs.* N.

therefore, the detection of RASSF1A gene methylation level in breast tissue or blood may be practical for distinguishing benign lesions from malignant lesions to some extent.

However, there were some limitations in this study. Firstly, there were only three major databases which we identified studies. And only 7 articles meet our inclusion criteria and the sample size of included studies was small. They might affect the effectiveness of our analysis to some extent. In addition, some of the included studies do not provide favourable data, including classification of breast cancer, tumor TNM staging, Patient's age, so we are unable to determine the impact of these factors on RASSF1A methylation in breast benign and malignant lesions. The further big data analysis about it is required. Last but not least, publication bias was a concern, for the journals tend to publish positive results. Those omitting unpublished research, which had negative results, might lead to exaggeration of the results in our study.

In summary, the present study showed that hyper-methylation of RASSF1A in cfDNA circulating in plasma or tissue may be a potential biomarker for diagnosis of breast cancer. These findings could let us find a simple and accessible way to measure DNA methylation and help identify women in the early stages of breast cancer. In order to improve the accuracy of breast cancer diagnosis, we need to combine other biomarkers for comprehensive analysis. But for these limitations existing, there is an urgently need to do large scale studies to determine whether hyper-methylation of RASSF1A can provide prognostic information in breast cancer, and whether it can make a different effect on different types of breast malignant lesions. Besides, different research platforms, sample types, ethnicities and other factors should be taken into consideration and analyzed by multi-level statistical analysis.

Conclusion

This meta-analysis confirms that hyper-methylation of RASSF1A

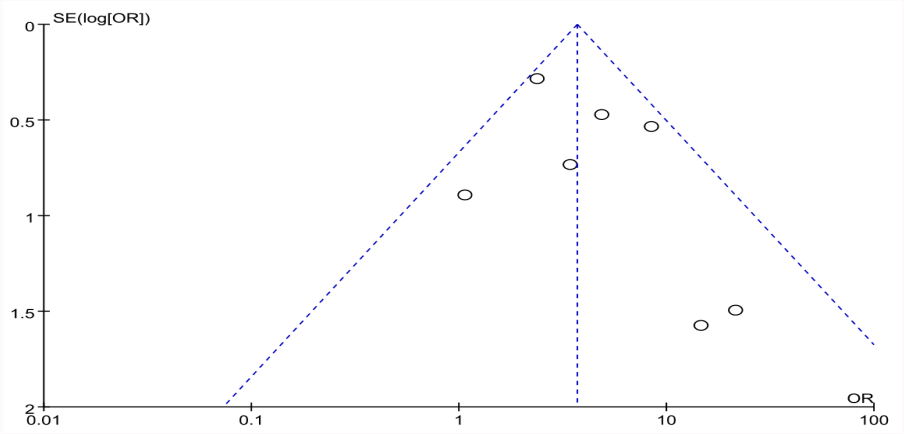

Figure 6: Funnel plot of BC *vs.* BBL.

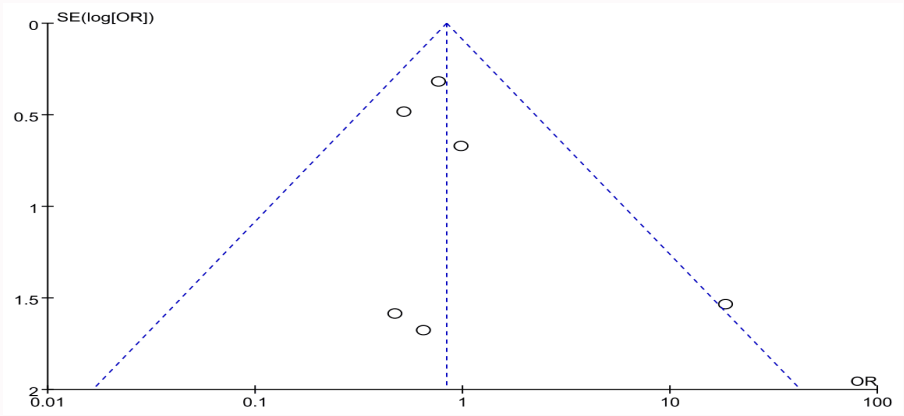

Figure 7: Funnel plot of BBL *vs.* N.

is closely related to breast tumor. And it is of significance in differentiating benign breast lesions from malignant breast lesions.

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Authors Contribution

Ying Hua Yu conceived the study. Xin Ling Deng and Xue Yan Chen designed and coordinated it. Xin Ling Deng and Chao Si Qian performed the statistical collection and analysis. Gui Ting Tang, Xin Ling Deng, Chao Si Qian and Xue Yan Chen drafted the manuscript. Li Ying Feng, Ying Hua Yu amended it. All authors participated in the review and revision of the manuscript. The final paper was composed, read and approved by all authors.

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