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Original Article

Prognostic and clinicopathological significance of microRNA-21 overexpression in breast cancer: a meta-analysis

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Abstract: Recent studies have highlighted the role of microRNA-21 (miR-21) as a prognostic biomarker of breast cancer. However, controversy still remains. The present study aimed to summarize available evidences and obtain a more precise estimation of a prognostic role of miR-21 in breast cancer patients. All eligible studies were searched from PubMed and EMBASE through multiple search strategies. Data were extracted from studies comparing survival in breast cancer patients having higher miR-21 expression with those having lower expression. A meta-analysis was performed to clarify prognostic role of miR-21 in patients with breast cancer. Subgroup analysis was also performed according to patients' ethnicity. A total of 6 eligible articles comprising 951 cases were selected for this meta-analysis. The combined hazard ratios (HRs) and 95% confidence intervals (95% *CI*s) for overall survival (OS) were 2.11 (1.09-4.08) and for disease free survival (DFS) was 1.6 (1.30-1.96). Subgroup analysis indicated high miR-21 expression was significantly associated with worse *OS* in Asian patients (*HR* = 4.39, 95% *CI*: 2.47-7.80) but not in non-Asian patients (*HR* = 1.18, 95% *CI*: 0.81-1.70). Sensitivity analysis revealed results of this meta-analysis were robust. Odds ratios (ORs) showed that miR-21 expression was closely associated with estrogen receptor (ER), progesterone receptor (PR), lymph node metastasis, histological grade, Her2/neu. The pooled *OR*s and 95% *CI*s were 0.53 (0.35-0.80), 0.49 (0.32-0.74), 2.32 (1.54-3.50), 2.44 (1.58-3.75), 4.29 (2.34-7.85), respectively. Our results indicated that elevated miR-21 expression could potentially predict poor survival in patients with breast cancer.

Keywords: MicroRNA-21, breast cancer, prognosis, biomarker, meta-analysis

Introduction

Breast cancer is one of the most common malignancies in women, which is a significant health problem ranking as a leading cause of cancer-related deaths among women around the world. The morbidity rate of breast cancer has gradually increased in recent decades [1-3] and 1.38 million individuals worldwide are affected every year [4]. Despite the extensive use of adjuvant systematic therapies such as radiotherapy, chemotherapy, hormone treatment, and biological therapy, the prognosis is still poor. More than 20% of patients with early breast cancer could develop incurable metastatic disease eventually [5, 6]. Therefore, factors to effectively evaluate the patients' survival outcome are needed urgently.

To date, there are a number of independent prognostic factors identified in clinical management of breast cancer, including tumor size, histological grade, nodal status, and patient age [7-9]. However, breast cancer is a heterogeneous disease with multiple factors involved in, and alterations in molecular mechanisms may affect tumor growth and progression, thereby the prognostic value of clinicopathological parameters is potentially limited [10]. Nowadays, a variety of potential prognostic biomarkers are being studied and applied in basic and clinical research, such as HER-2/neu,

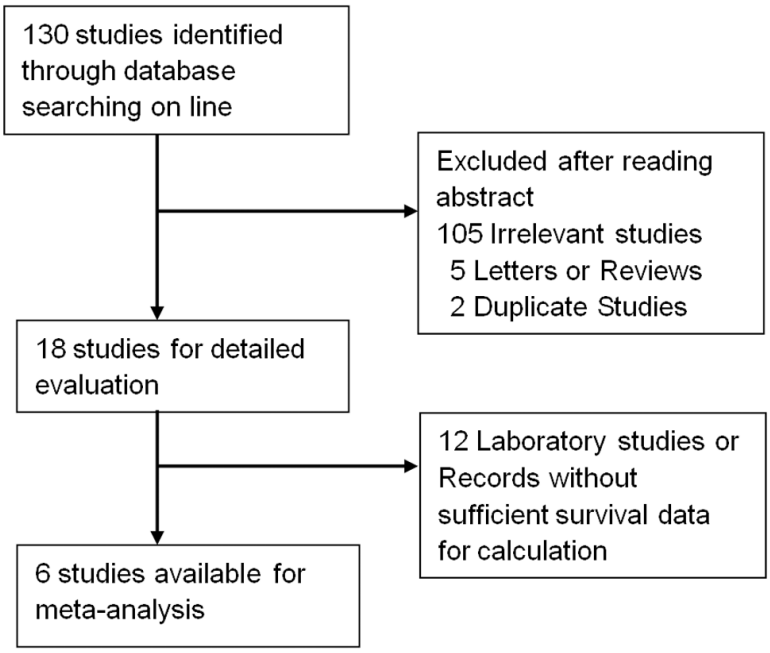


Figure 1. Flow diagram of the study selection process.

estrogen receptor (ER), progesterone receptor (PR), cyclin E, p53, bcl-2, vascular endothelial growth factor, urokinase-type plasminogen activator-1. However, no single biomarker was able to predict those patients with the best (or worst) prognosis due to its the discriminant value on the complex , heterogenetic disease [10].

MicroRNAs (miRNAs) are endogenous, small non-coding RNAs molecules with a length of 18-25 nucleotides. They were first reported in 1993 [11]. MicroRNA could identify post transcriptional gene regulators that paired to complementary sequences in the 3'untranslated region (3'UTR) of target mRNAs, and regulate protein-coding genes expression by mRNA degradation or translational repression [12]. These miRNAs may regulate the translation of specific protein-coding genes [13, 14].

Recent studies have revealed that microRNA-21 (miR-21) is elevated in many kinds of cancer, including breast cancer [15, 16], colorectal cancer [17, 18], lung cancer [19, 20], pancreatic cancer [21, 22], prostate cancer [23], gastric cancer [23], glioblastoma [24], and is recognized a potential oncomicroRNA. Many studies have shown that overexpression of miR-21 could increase cell growth, migration, invasion, survival [25, 26] and inhibit apoptosis [27]. Studies also demonstrate that miR-21 can modulate several tumor suppressor genes, including phosphatase and tensin homolog (PTEN) [28], tropomyosin 1 (TPM1) [29], programmed cell death 4 (PDCD4) [30], and may play a role in cancer invasion and metastasis.

Prognostic role of microRNA-21 (miR-21) in breast cancer has been highlighted in some studies. However, controversy still remains. In this study, we aim to perform a meta-analysis to evaluate and predict the overall risk of high miR-21 expression for survival prognosis and clinicopathological features in breast cancer patients and discuss the challenges of miR-21 as a possible prognosis biomarker in breast cancer.

Material and methods

This meta-analysis was carried out in accordance with the guidelines of the meta-analysis of the Observational Studies in Epidemiology group (MOOSE) [31].

Search strategy

PubMed and EMBASE were searched for the last time on May 15, 2014. The search strategy included the following keywords: miR-21, microRNA-21, breast Cancer, breast carcinoma, prognostic and prognosis. A manual review of the references of relevant publications was also performed to obtain additional studies.

Study selection

Studies were considered eligible if they met the following criteria: (i) they had to study the patients with breast cancer; (ii) they had to detect the expression of miR-21 in tissue, serum, or other clinical samples; (iii) they had to investigate the survival outcome or the correlation between miR-21 expression and the clinical variables. (iv) the method for detecting miR-

Table 1. Clinical characteristics of studies included in the meta-analysis

First author	Year	Population	Study Design	Number	Materials	Stage	Method	Cut-off	Survival analysis	Hazard Ratios	Follow-up, months	Quality score
Yan	2008	China	R	113	Tumor	I-III	qRT-PCR	Mean	OS	Reported	66.2 (10.4-81.0)	7
Qian	2008	Italy	R	301	Tumor	I-IV	qRT-PCR	Median	OS	Reported	86.2 (8.0-108)	6
										Reported	86.2 (8.0-108)	
Lee	2011	Korea	R	109	Tumor	I-III	qRT-PCR	Mean	OS	Reported	median 54	7
									DFS	SC	median 54	
OTA	2011	Japan	R	291	Bone marrow	--	qRT-PCR	5.84	OS	SC	61 (2-90)	7
									DFS	Reported	61 (2-90)	
Markou	2013	Greece	R	112	Tumor	I-IV	qRT-PCR	Median	OS	Reported	84 (10-149)	7
									DFI	Reported	84 (10-149)	
Walter	2011	the USA	R	25	Tumor	I-IV	qRT-PCR	Median	OS	SC	median 35.5	6

The studies included here are all retrospective cohort studies with different groups of patients. qRT-PCR, quantitative real-time PCR; --, not mentioned; OS, overall survival; DFS, disease-free survival; DFI, disease-free interval; SC, survival curve.

21 must be same. Articles were excluded based on any of the following reasons: (i) review articles, laboratory articles or letters; (ii) They were described the survival outcome of other tumors or other markers; (iii) They lacked key information for calculation with methods developed by Parmar, Williamson , and Tierney [32-34]; (iv) They were not English; (v) the articles from one author and the studies brought into the repeated samples from the same patients when a study already included.

The information such as titles, abstracts, full texts and reference lists of all of the identified reports was carefully identified in duplicate by two investigators (Pan and Mao). These extracted articles were double checked by Geng. Disagreements were resolved by discussion among all authors in this paper. All the data were consensus. The references from the relevant literatures, including all the identified studies, reviews and editorials, were also reviewed manually.

Quality assessment

The quality of all the studies included was systematically evaluated according to a critical review checklist of the Dutch Cochrane Centre proposed by MOOSE [31, 35]. The current checklist included the following key points: (i) clear definition of study population and origin of country; (ii) clear definition of type of carcinoma; (iii) clear definition of study design; (iv) clear definition of outcome assessment; (v) clear definition of measurement of miR-21; (vi) sufficient period of follow-up. Any study without mentioning these points is excluded so as not to compromise the quality of the meta-analysis.

Data extraction

Data were extracted from each study according to the before-mentioned selection criteria. The primary information was collected in a form: first author's name, year of publication, country of origin, ethnicity, total number of cases, follow-up time, multivariate analysis, univariate analysis, kaplan-meier survival analysis, *P* value, 95% *CI*, and hazard ratios independently. Other extracted data elements were included as the following: age, gender, TNM stage, lymph status, histological classification, method of detecting miR-21, Estrogen Receptor (ER), Progesterone Receptor (PR), Her2/neu. An *HR* of > 1 was considered significant association with a poorer outcome. If only survival curves were available, data were extracted from the graphical survival plots and HR was then estimated according to the previously described method [32, 34].

Statistical methods

Statistics were conducted as described previously [36]. Hazard Ratios (*HR*s) and 95% confidence intervals (95% *CI*s) were calculated for each study. But some studies did not list the *HR*s or 95% confidence intervals (95% *CI*s) directly, only giving Kaplan-Meier survival curves instead. The necessary statistics were calculated using software developed by Matthew Sydes and Jayne Tierney (Medical Research Council Clinical Trials Unit, London, UK) [34].

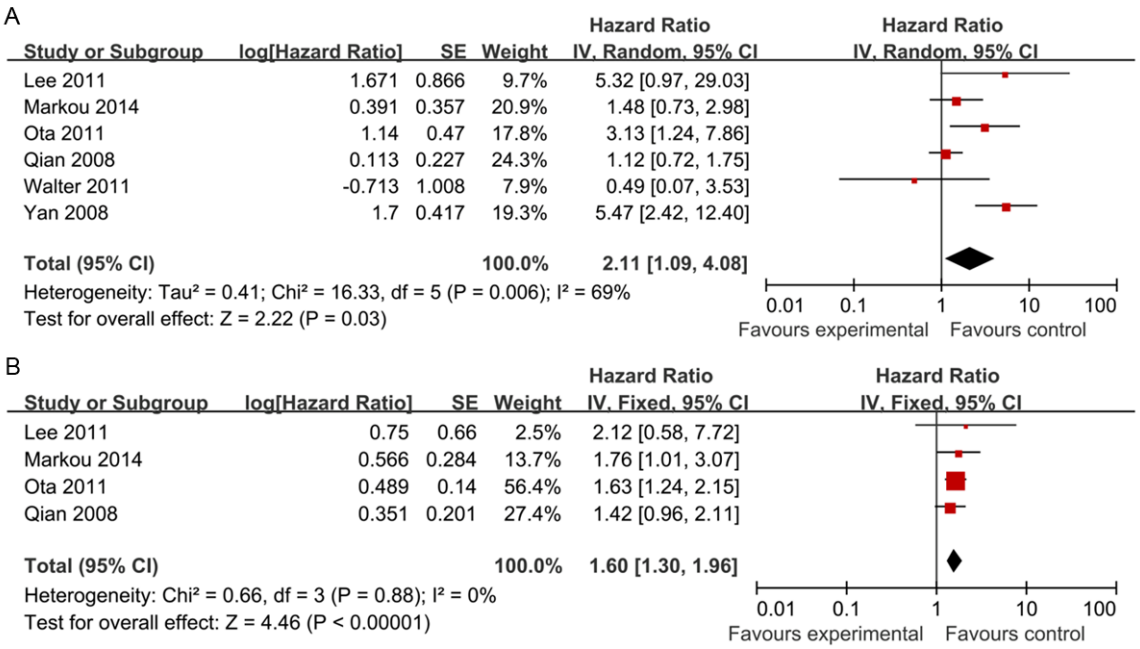


Figure 2. Forest plot of studies evaluating hazard ratio (HR) for the association of high miR-21 expression with overall survival (OS) (A) and disease-free survival (DFS) (B) in patients with breast cancer patients.

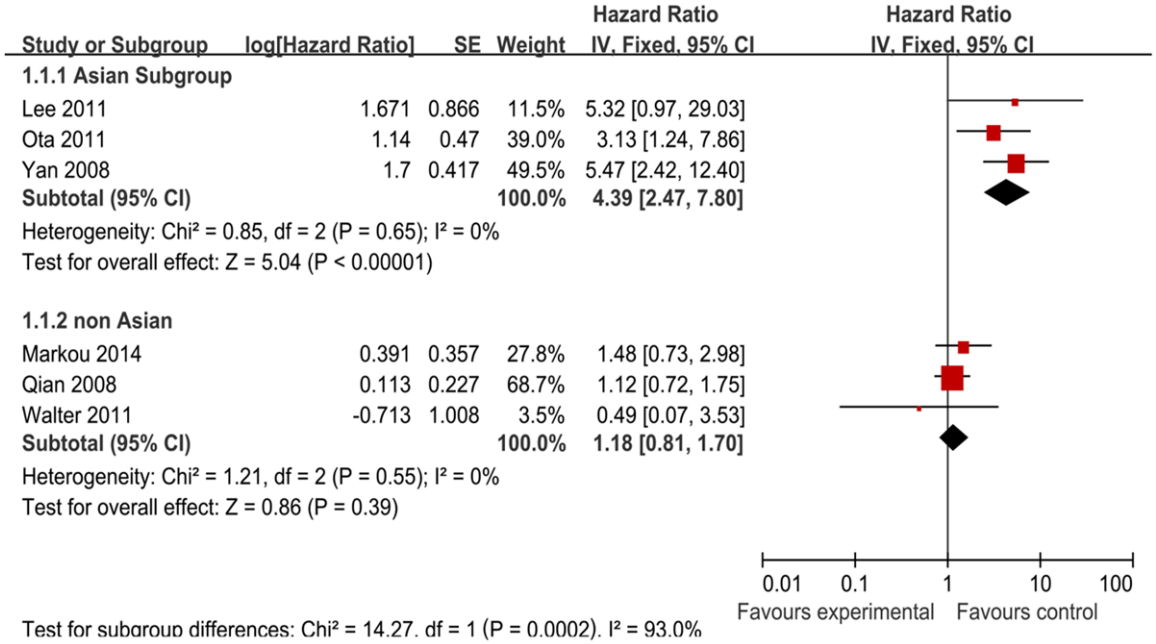


Figure 3. Forest plot of studies evaluating hazard ratio (HR) for the association of high miR-21 expression with overall survival (OS) stratified according to source of ethnicity.

Forest plots were used to estimate the aggregation effect of miR-21 expression on survival outcome (OS and DFS) and the correlation between miR-21 expression and clinical variables. Subgroup analysis of pooled hazard ratios was performed subsequently by ethnicity. For each meta-analysis performed, Cochran's Q test and Higgins I-squared statistic were carried out to assess the between-study heterogeneity, and heterogeneity was consid-

Table 2. Subgroup analysis of pooled *HR*s of breast cancer patients with high miR-21 expression for OS

Subgroup	Number of studies	Number of patients	*HR* (95% *CI*)	*P* value	Heterogeneity	
					I^2 (%)	*P* value
Patients' ethnicity						
Asian	3	513	4.39 (2.47-7.80)	0.00001	0%	0.65
non-Asian	3	438	1.18 (0.81-1.70)	0.39	0%	0.55
Number of patients						
>200	2	592	1.72 (0.64-4.65)	0.28	74%	0.05
<200	4	359	2.4 (0.89-6.44)	0.08	67%	0.03

Table 3. *HR*s (95% *CI*) of sensitivity analysis on OS for the meta-analysis

Study omitted	Estimated *HR*	Low value of 95% *CI*	High value of 95% *CI*	*P* value	Heterogeneity	
					I^2 (%)	*P* value
Yan 2008	1.42	1.01	1.99	0.04	46%	0.12
Qian 2008	2.6	1.26	5.36	0.01	57%	0.05
Lee 2011	1.91	0.96	3.82	0.07	73%	0.006
OTA 2011	1.94	0.9	4.18	0.09	72%	0.006
Walter 2011	2.39	1.2	4.74	0.01	73%	0.005
Markou 2013	2.32	0.97	5.54	0.06	75%	0.003
Combined	2.11	1.09	4.08	0.03	69%	0.006

Table 4. *HR*s (95% *CI*) of sensitivity analysis on DFS for the meta-analysis

Study omitted	Estimated *HR*	Low value of 95% *CI*	High value of 95% *CI*	*P* value	Heterogeneity	
					I^2 (%)	*P* value
Qian 2008	1.67	1.31	2.13	0.0001	0%	0.91
Lee 2011	1.59	1.29	1.95	0.0001	0%	0.79
OTA 2011	1.56	1.14	2.13	0.006	0%	0.74
Markou 2013	1.57	1.26	1.96	0.0001	0%	0.77
Combined	1.6	1.3	1.96	0.00001	0%	0.88

ered significant for $P < 0.1$. A random-effects model with DerSimonian and Laird method were then applied to calculate the poor *HR*, if heterogeneity was observed ($P < 0.1$), Otherwise, the fixed-effects model was used [37]. Pooled $HR > 1$ indicated poor prognosis for the groups with elevated miR-21 expression and was considered statistically significant if the 95% *CI* did not overlap 1. Sensitivity analysis was applied by excluding each single study. Finally, publication bias was evaluated using the funnel plot, Begg's test [38], Egger's test [39]. $P > 0.05$ was considered that there was no potential publication bias in the meta-analysis. All analyses were executed using the software Review Manager 5.1 (The Cochrane Collaboration, Oxford, United Kingdom) and Stata 12.0 (http://www.stata.com/; Stata Corporation, College Station, Texas, USA).

Results

Characteristics of the studies included in Meta-analysis

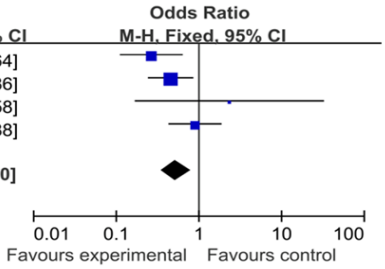
As shown in **Figure 1**, 130 records for miR-21 and breast cancer were identified in Pubmed and EMBASE. After reviewing these abstracts, 112 studies were excluded due to their irrelevance to the current analysis, letters, reviews and duplicate studies. Furthermore, 12 potential studies were excluded, due to laboratory studies or records without sufficient survival data for calculation. Therefore, 6 eligible articles were enrolled in this meta-analysis [40-45].

These eligible studies were published between 2008 and 2013. One studies evaluated patients from China, one evaluated patients from Italy, one evaluated patients from Korea, one evaluated patients from Japan, one evaluated patients from Greek, and one evaluated patients from the United States of America. These studies included a total of 951 patients with a mean number of 158.5 patients per study. These six eligible studies were all retrospective cohort studies. The method of miR-21 detection was all quantitative real-time polymerase chain reaction (qRT-PCR). MiR-21 expression levels were measured in tumor tissue or bone marrow. The mean or medium length of follow-up ranged

A

Study or Subgroup	Experimental Events	Total	Control Events	Total	Weight	Odds Ratio M-H, Fixed, 95% CI
Lee 2011	14	73	17	36	30.2%	0.27 [0.11, 0.64]
Ota 2011	24	194	23	97	44.2%	0.45 [0.24, 0.86]
Walter 2011	2	3	6	13	1.2%	2.33 [0.17, 32.58]
Yan 2008	30	57	31	56	24.3%	0.90 [0.43, 1.88]
Total (95% CI)		**327**		**202**	**100.0%**	**0.53 [0.35, 0.80]**
Total events	70		77			

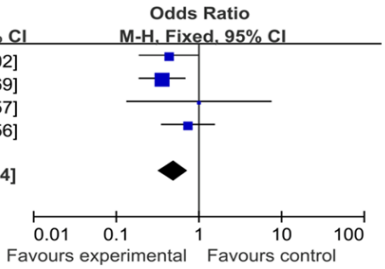
Heterogeneity: Chi² = 5.77, df = 3 (P = 0.12); I² = 48%
Test for overall effect: Z = 3.04 (P = 0.002)



B

Study or Subgroup	Experimental Events	Total	Control Events	Total	Weight	Odds Ratio M-H, Fixed, 95% CI
Lee 2011	14	65	17	44	24.9%	0.44 [0.19, 1.02]
Ota 2011	17	166	29	120	47.3%	0.36 [0.19, 0.69]
Walter 2011	3	6	5	10	2.9%	1.00 [0.13, 7.57]
Yan 2008	33	65	28	48	24.8%	0.74 [0.35, 1.56]
Total (95% CI)		**302**		**222**	**100.0%**	**0.49 [0.32, 0.74]**
Total events	67		79			

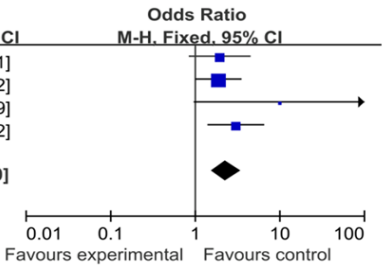
Heterogeneity: Chi² = 2.57, df = 3 (P = 0.46); I² = 0%
Test for overall effect: Z = 3.37 (P = 0.0007)



C

Study or Subgroup	Experimental Events	Total	Control Events	Total	Weight	Odds Ratio M-H, Fixed, 95% CI
Lee 2011	17	47	14	62	26.1%	1.94 [0.84, 4.51]
Ota 2011	25	117	22	174	47.1%	1.88 [1.00, 3.52]
Walter 2011	10	16	1	7	1.8%	10.00 [0.96, 104.49]
Yan 2008	42	64	19	49	25.0%	3.01 [1.39, 6.52]
Total (95% CI)		**244**		**292**	**100.0%**	**2.32 [1.54, 3.50]**
Total events	94		56			

Heterogeneity: Chi² = 2.54, df = 3 (P = 0.47); I² = 0%
Test for overall effect: Z = 4.02 (P < 0.0001)



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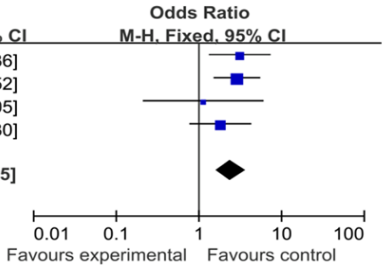
Study or Subgroup	Experimental Events	Total	Control Events	Total	Weight	Odds Ratio M-H, Fixed, 95% CI
Lee 2011	18	42	13	67	22.5%	3.12 [1.32, 7.36]
Ota 2011	21	74	26	215	37.4%	2.88 [1.50, 5.52]
Walter 2011	9	17	4	8	10.1%	1.13 [0.21, 6.05]
Yan 2008	49	85	12	28	30.0%	1.81 [0.77, 4.30]
Total (95% CI)		**218**		**318**	**100.0%**	**2.44 [1.58, 3.75]**
Total events	97		55			

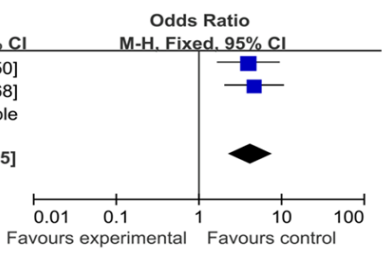
Heterogeneity: Chi² = 1.83, df = 3 (P = 0.61); I² = 0%
Test for overall effect: Z = 4.06 (P < 0.0001)



E

Study or Subgroup	Experimental Events	Total	Control Events	Total	Weight	Odds Ratio M-H, Fixed, 95% CI
Lee 2011	19	41	12	67	52.6%	3.96 [1.65, 9.50]
Ota 2011	15	32	22	138	47.4%	4.65 [2.03, 10.68]
Walter 2011	8	16	0	0		Not estimable
Total (95% CI)		**89**		**205**	**100.0%**	**4.29 [2.34, 7.85]**
Total events	42		34			

Heterogeneity: Chi² = 0.07, df = 1 (P = 0.79); I² = 0%
Test for overall effect: Z = 4.72 (P < 0.00001)



F

Study or Subgroup	Experimental Events	Total	Control Events	Total	Weight	Odds Ratio M-H, Fixed, 95% CI
Lee 2011	11	37	20	72	45.7%	1.10 [0.46, 2.63]
Walter 2011	7	15	2	5	7.7%	1.31 [0.17, 10.26]
Yan 2008	36	58	25	55	46.6%	1.96 [0.93, 4.16]
Total (95% CI)		**110**		**132**	**100.0%**	**1.52 [0.88, 2.62]**
Total events	54		47			

Heterogeneity: Chi² = 0.99, df = 2 (P = 0.61); I² = 0%
Test for overall effect: Z = 1.51 (P = 0.13)

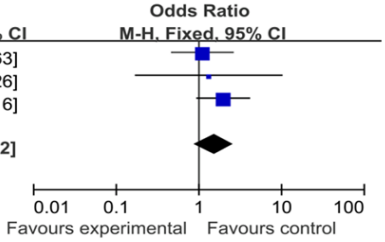


Figure 4. Forrest plot of odds ratios (ORs) for the association of miR-21 expression with clinicopathological features in breast cancer. A. ORs with corresponding 95% CIs of miR-21 expression with Estrogen Receptor (ER). B. ORs with corresponding 95% CIs of miR-21 expression with Progesterone Receptor (PR). C. ORs with corresponding 95% CIs of miR-21 expression with lymphnode metastasis. D. ORs with corresponding 95% CIs of miR-21 expression with Histologic grade. E. ORs with corresponding 95% CIs of miR-21 expression with HER2/neu. F. ORs with corresponding 95% CIs of miR-21 expression with age.

Table 5. Meta-analysis of miR-21 expression classified by clinical features

Clinicopathological features	Number of studies	Number of patients	Model	Pooled *OR* (95% *CI*)	*P* value	Heterogeneity	
						I^2 (%)	*P* value
ER (postive vs. negative)*	4	529	Fixed	0.53 (0.35-0.80)	0.002	48%	0.12
PR (postive vs. negative)*	4	524	Fixed	0.49 (0.32-0.74)	0.0007	0%	0.46
Lymph node metastasis (postive vs. negative)	4	536	Fixed	2.32 (1.54-3.50)	0.0001	0%	0.47
Histologic grade (G3 vs. G1, 2)	4	536	Fixed	2.44 (1.58-3.75)	0.0001	0%	0.61
HER2/neu (postive vs. negative)	3	294	Fixed	4.29 (2.34-7.85)	0.00001	0%	0.79
Age (> median vs. < median)	3	242	Fixed	1.52 (0.88-2.62)	0.13	0%	0.61

*ER: Estrogen Receptor; PR: Progesterone Receptor.

from 35.5 to 86.2 months. Characteristics of the eligible studies are summarized in **Table 1**.

Methodological quality of the studies

The qualities of 6 eligible studies included in our meta-analysis were assessed according to the Newcastle-Ottawascale (NOS). The NOS contained eight items of methodology, which were categorized into the three dimensions of selection, comparability, and outcome. It was used to assess the quality of non-randomized studies in meta-analysis. For quality, scores ranged from 0 (lowest) to 9 (highest), and studies with scores of more than 5 were identified as high quality. All these 6 eligible studies gained more than 5 scores in methodological assessment, indicating that they were of high quality relatively (**Table 1**).

Correlation between miR-21 expression and survival outcome (OS and DFS)

The meta-analysis was performed on 6 studies [40-45] assessing the association of miR-21 expression with OS and DFS. As shown in **Figure 2**, the pooled *HR* for OS was 2.11 (95% *CI*: 1.09-4.08; $Z = 2.22$; $P = 0.03$) with heterogeneity ($I^2 = 69.0\%$, $P = 0.006$). In the case of heterogeneity, a random-effects model was used. The pooled *HR* of four studies [41-43, 45] for DFS was 1.60 (95% *CI*: 1.30-1.96; $Z = 4.46$; $P < 0.00001$) with no heterogeneity ($I^2 = 0\%$, $P = 0.88$), and the fixed efforts model was used. It suggested that high miR-21 expression was statistically significant with the worse prognosis of breast cancer and high miR-21 expression was a valuable prognostic factor in breast cancer.

Subgroup analysis

Moreover, we carried out subgroup analysis by the patients' ethnicity. The results showed that there was significant relation between high miR-21 expression and OS, especially in Asian countries (**Figure 3**) (**Table 2**). The combined HR of Asian studies [40, 42, 43] for OS was 4.39 (95% *CI*: 2.47-7.80; $Z = 5.04$; $P < 0.00001$), while the combined *HR* of the other yielded non-Asian studies [41, 44, 45] was 1.18 (95% *CI*: 0.81-1.70; $Z = 0.86$; $P = 0.39$). As a result of this subgroup analysis, the heterogeneity was both eliminated. We also tried to use the other grouping term to examine the prognostic role of miR-21, such as number of patients (**Table 2**). No results could give clinical significance.

Sensitivity analysis

The conclusions remained similar when a single study involved in the meta-analysis was removed each time to reflect the influence of the rest data-set on the pooled *HR*s. The summary *HR* for OS ranged from 1.42 (95% *CI*: 1.01-1.99) after excluding the study of Yan [40] to 2.60 (95% *CI*: 1.26-5.36) after excluding the study of Qian [41] (**Table 3**). The summary *HR* for DFS ranged from 1.56 (95% *CI*: 1.14-2.13) after excluding the study of OTA to 1.67 (95%

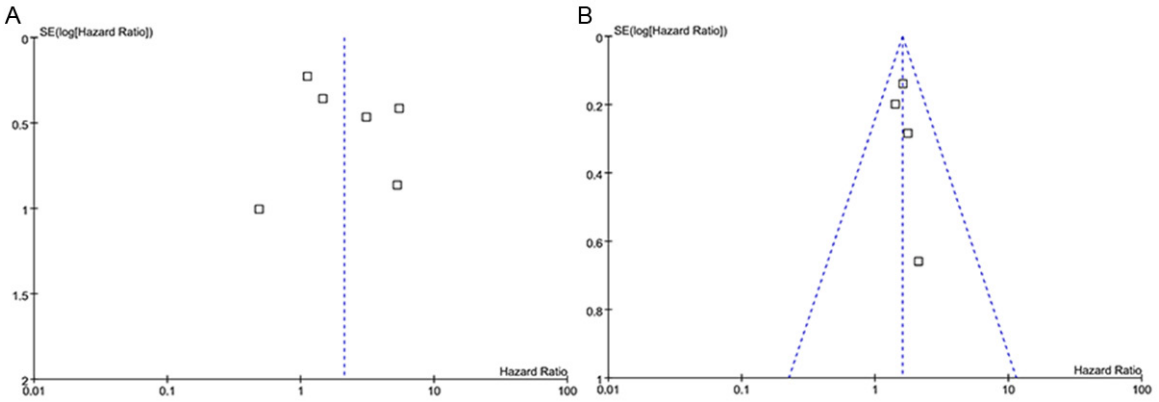


Figure 5. A: Funnel Plots of elevated miR-21 expression and OS in patients with breast cancer. B: Funnel Plots of elevated miR-21 expression and DFS in patients with breast cancer.

CI: 1.31-2.13) after excluding the study of Qian (**Table 4**).

Correlation between miR-21 expression and clinical characteristics

The studies which referred the association between miR-21 expression and some clinical characteristics (ER, PR, lymph node metastasis, histological grade, Her2/neu, and age) have been combined to calculate the Odds ratios (*ORs*). Four studies [40, 42-44] evaluated the correlation between miR-21 expression and ER and PR. The pooled ORs were 0.53 (95% *CI:* 0.35-0.80, $P = 0.002$) with no heterogeneity ($I^2 = 48\%$, $P = 0.12$) and 0.49 (95% *CI*: 0.32-0.74, $P = 0.0007$) with no heterogeneity ($I^2 = 0\%$, $P = 0.46$) respectively, indicating that miR-21 expression was negatively related to ER and PR (**Figure 4A** and **4B**) (**Table 5**). We also assessed the correlation between miR-21 expression and lymphnode node metastasis and Histologic grade. The pooled *ORs* were 2.32 (95% *CI*: 1.54-3.50, $P = 0.0001$) and 2.44 (95% *CI*: 1.58-3.75, $P = 0.0001$) respectively (**Figure 4C** and **4D**) (**Table 5**), which suggested that high miR-21 expression was associated with metastasis and histologic grade of breast cancer. Moreover, the association between miR-21 expression and Her2/neu was identified, with the pooled *OR* 4.29 (95% *CI*: 2.34-7.85, $P = 0.00001$), suggesting that high miR-21 expression was related to Her2/neu. Meanwhile, there was no significant association between high miR-21 expression and age (**Figure 4E**). All these results could be reviewed in **Table 5**.

Assessment of publication bias

In the funnel plot analysis, the shape of the funnel plot seemed symmetrical for both OS and DFS. Egger's test and Begg's test were used to examine publication bias. There were no significant publication biases in the meta-analysis of miR-21 prediction value for OS (Egger's test, $P = 0.429$; Begg's test, $P = 0.999$) and DFS (Egger's test, $P = 0.521$; Begg's test, $P = 0.308$) respectively (**Figure 5**).

Discussion

Alteration of biological markers in tumor tissues plays an important role in predicting the prognostic value of the breast cancer patients [10]. Breast cancer is a malignant disease and is known to be quite complex and heterogeneous in development, progress and response to treatment. As a result, biomarkers available such as ER, PR, HER2, could not reflect the whole prognostic significance for breast cancer patients. Therefore, besides ER, PR, and HER2, it is important to find out new prognostic biomarkers for patients with breast cancer.

Recently, numbers of studies are emerging that microRNA could be considered as revolutionary sources of biomarkers for cancer prognosis [46]. MiR-21, known as a potential oncogenic role, is one of the most commonly observed aberrant miRNAs in a variety of cancers [15-24]. And it could be measured stably and easily in tumor tissues, formalin-fixed paraffin-embedded tissues and blood circulation [14, 47]. In breast cancer, some studies found that miR-21

was significantly associated with patients' survival [40, 43]. However, insignificant results of miR-21 were also observed in other studies [41]. Meta-analysis is a systematical approach applied widely to the evaluation of prognostic indicators in different studies. There is no consensus on the association between high miR-21 expression and poor survival prognosis in patients with breast cancer currently, nevertheless the prognostic role of miR-21 has been discussed in head and neck squamous cell carcinoma (HNSCC), non-small-cell lung cancer (NSCLC) and colorectal cancer (CRC) [36, 48, 49]. Thus, a quantitative meta-analysis was performed to determine the association between miR-21 expression and the survival prognosis and clinicopathological features in breast cancer patients.

This meta-analysis revealed that elevated miR-21 expression did predict poor survival in patients of breast cancer. The pooled HR for OS was 2.11 (95% *CI*: 1.09-4.08, $P = 0.03$) with heterogeneity ($I^2 = 69.0\%$, $P = 0.006$). The differences of HRs were found to be statistically significant, but significant heterogeneity was also observed among the studies. Then we used random effects model to analyze the data, however, heterogeneity was still a potential problem to affect meta-analysis results. Moreover, the result remained similar in a sensitivity analysis when a single study was removed each time. After subgroup analysis by the patients' ethnicity, the heterogeneity was eliminated both in Asian studies and non-Asian studies. The HR for Asian countries was 4.39 (95% *CI*: 2.47-7.80, $P < 0.00001$). It suggested that the miR-21 expression played significant prognostic role on breast cancer in Asian group. Meanwhile, the combined HR for non-Asian group was 1.18 (95% *CI*: 0.81-1.70, $P = 0.39$), which suggested that the miR-21 had no significant effect to predict survival outcome in non-Asian group. As a result, the heterogeneity in this meta-analysis might be explained by the patients' ethnicity and miR-21 expression could be racial different as a prognostic factor. The pooled HR for DFS was 1.60 (95% *CI*: 1.30-1.96, $P < 00001$) with no heterogeneity ($I^2 = 0\%$, $P = 0.88$). The difference was statistically significant, though the HR was not so strong. As referred in Hayes [50], a prognostic factor with $HR > 2$ is considered strongly predictive. Combining the results for OS and DFS, it may suggest that miR-21 expression in breast cancer patients could predict their prognosis practically, especially in the Asian population.

Furthermore, the correlation between miR-21 expression and clinical characteristics was calculated. There was no significant association between high miR-21 expression and age, while ORs for ER, PR, lymph node metastasis, histological grade, Her2/neu, were significant. Thus, elevated miR-21 expression was closely associated with worse clinical characteristics.

Still some limitations existed in this meta-analysis. First, there are only six studies included in this meta-analysis and the pooled HR was calculated on the basis of these 6 studies with a small sample size of 951 patients. Second, the cut-off values of miR-21 were different in the selected studies. Median and mean values were often chosen as the cut-off values. However, there was no final conclusion to confirm how high was considered high. Third, miR-21 was detected in tumor tissue and bone marrow but few in serum or plasma in the selected studies. Circulating prognostic markers were more likely to be accepted than tissue markers and it could be detected before surgery and be monitored throughout the lives of the cancer patients. Fourth, this meta-analysis did not evaluate the prognostic value of a combination of miR-21 and other miRNA markers for breast cancer cases. Fifth, some studies didn't provide HRs, and the data were extracted from survival curves, which might be of less credibility than direct analysis on HRs.

Publication bias is a major concern for meta-analysis. In our research, neither Egger's test nor Begger's test showed evidence of publication bias; however, bias might still have occurred. It should be noted that positive results tend to be accepted by journals, while negative results are often rejected or not even submitted.

In conclusion, our meta-analysis showed that elevated miR-21 expression was significantly associated with poor survival in breast cancer patients and might potentially predict the poor survival in patients with breast cancer. More multi-center clinical investigations with larger sample size should be conducted to further confirm these results.

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Disclosure of conflict of interest

None.

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