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*Original Article*

# Prognostic role of miR-200c in various malignancies: a systematic review and meta-analysis

Ke-Cheng Zhang*, Hong-Qing Xi*, Jian-Xin Cui*, Wei-Song Shen, Ji-Yang Li, Bo Wei, Lin Chen

*Department of General Surgery, Chinese People's Liberation Army General Hospital, 28 Fuxing Road, Beijing 100853, China. *Equal contributors.*

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**Abstract:** MiR-200c expression is dysregulated in various malignancies and may predict the survival of patients with cancer, although the results of different studies conflict. Therefore, we conducted a meta-analysis to resolve this discrepancy. We queried the PubMed and Embase using multiple search strategies. Data were extracted from studies comparing overall survival and progression-free survival in patients with cancer with high and low levels of miR-200c expression. Fixed and random models were used where appropriate. A combined hazards ratio (HR) was calculated to estimate the association of high levels of miR-200c with survival. We selected 16 studies of 1485 participants for our final meta-analysis. Upregulated expression of miR-200c predicted significantly worse overall survival in patients with cancer (HR 1.51; 95% confidence interval [CI] 1.06-2.16, $P = 0.023$). Subgroup analysis indicated that high levels of miR-200c was associated with decreased survival of Caucasians and patients with gynecological tumors with pooled HR values of 1.82 (95% CI 1.27-2.26, $P = 0.01$) and 3.23 (95% CI 1.11-9.38, $P = 0.032$), respectively. Because of the absence of apparent heterogeneity, the combined HRs were 1.69 (95% CI 1.24-2.30, $P = 0.001$) for squamous cell carcinoma and 1.91 (95% CI 1.40-2.59, $P < 0.001$) for samples from peripheral blood. Increased expression of miR-200c significantly associated with shorter progression-free survival of patients with cancer (HR 2.37; 95% CI 1.47-3.81, $P < 0.001$). Our meta-analysis indicates that the level of miR-200c expression predicted survival of patients with cancer, particularly for Caucasians and patients with gynecological cancer. Increased expression of miR-200c predicted shorter survival of patients with squamous cell carcinomas. Our findings indicate that monitoring the levels of miR-200c in blood may be useful for following tumor progression as well as patients' prognosis.

**Keywords:** Prognosis, miR-200c, cancers, survival, meta-analysis

## Introduction

MicroRNA (miRNAs) are a class of highly conserved, small (average length 22 nucleotides), and endogenously expressed noncoding RNAs that function as post-transcriptional regulators of gene expression. To date, more than 2,000 mature miRNAs have been identified [1], which can target huge amounts of human genes. Moreover, a single miRNA may target more than one sequence because of imperfect complementarity. This endows miRNAs with the potential to regulate diverse physiological processes including cellular differentiation [2], proliferation [3], and apoptosis [4]. Thus, altered expression of miRNAs has been related to diseases such as myocardial infarction [5], diabetes [6], and rheumatoid arthritis [7], and numerous studies also link the aberrant expression of miRNAs to human cancer [8-10].

We reviewed the association between miR-21 and colorectal cancer and demonstrated the miR-21 may serve as a diagnostic and prognostic marker for colorectal cancer [11]. Here, we focused on miR-200c because of its association with potential cancer-risk regions identified by transcriptome data for miR-21 [12]. MiR-200c is one of the five members of the miR-200 family, and its gene resides on chromosome 12p13. The expression of miR-200c was investigated in gastric [13], lung [14], prostate [15], and esophageal cancers [16] as well as others [17, 18].

Hurteau et al. [19] were the first to detect differential expression of miR-200c by certain

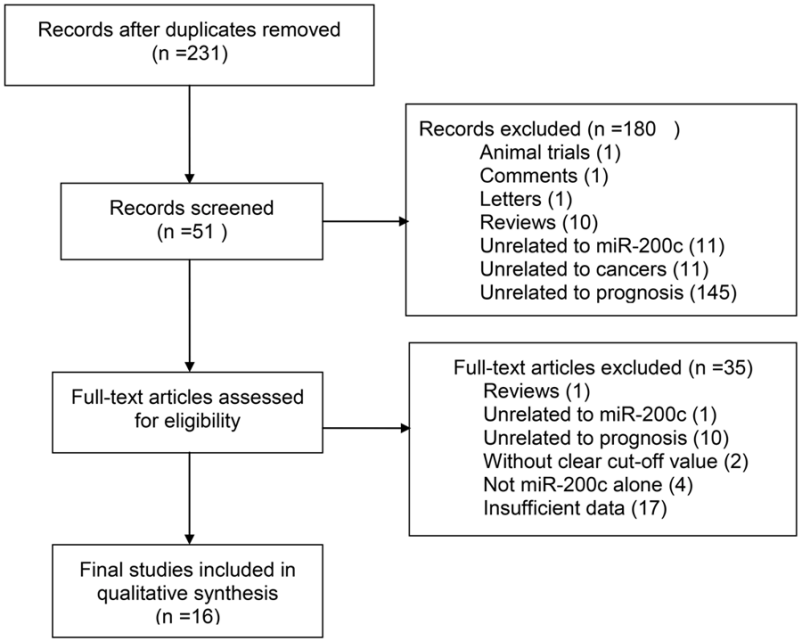


**Figure 1.** Flow diagram showing the process for selecting eligible studies.

cancer cell lines and demonstrated that overexpression of miR-200c contributed to reduced or increased expression of ZEB1 or E-cadherin, respectively [20]. Subsequently, Iorio et al. [21] reported that miR-200c is among the most highly expressed miRNAs in ovarian cancers. Shimono et al. [22] demonstrated that tumor formation driven by human breast cancer stem cells is strongly suppressed by miR-200c, which modulates the expression of BMI1, a regulator of stem cell self-renewal. Park et al. [23] reported that miR-200c, acting through ZEB1 and ZEB2, regulates the epithelial-mesenchymal transition, which is essential for embryonic development and is involved in tumor metastasis and progression associated with clinical outcomes. The relationship between aberrant expression of miR-200c and the survival of patients with cancer has been extensively investigated [24, 25]. Because the results of these studies are inconsistent, it is difficult to implicate miR-200c in pathogenesis.

Therefore, the aim of our study was to conduct a meta-analysis to determine whether detection of miR-200c expression in human cancers predicts clinical outcomes. This comprehensive meta-analysis is the first, to our knowledge, to evaluate the relationship between miR-200c expression and survival in patients with cancer.

## Materials and methods

### *Search strategy*

We performed this meta-analysis according to the guidelines of the Meta-analysis of Observational Studies in Epidemiology group (MOOSE) [26]. We carefully and systematically searched the PubMed and Embase database (final search conducted Sept 1, 2014) with lower limit of Jan 1st, 1993. Only articles published in English were considered, and we excluded conference abstracts because of incomplete data. The main key words for retrieval were variably combined as follows:"microRNA-200c","miR-200c", "has-miR-200c", "overall survival", "prognosis", "cancer", "carcinoma", "tumor", and "neoplasm". Moreover, we manually screened the references of relevant studies and conference summaries to check for potentially relevant articles.

### *Criteria for selection*

To insure that our analyses were reliable and credible, eligible studies were selected according to the criteria as follows: (i) miR-200c expression in patients with cancer; (ii) expression levels of miR-200c in tumors or peripheral blood; and (iii) association between miR-200c and survival outcome. Three investigators (Zhang, Xi, and Cui) independently and manually scanned these articles and excluded those meeting any of the criteria as follows: (i) review articles, comments, and letters; (ii) non-English articles; (iii) focused on a set of miRNAs rather than miR-200c alone; (iv) lack of key information such as hazard ratio (HR), 95% confidence interval (CI), and *p* value or insufficient data to calculate HR and CI. Eligible studies met the above inclusion criteria. The senior reviewers (Chen and Wei) resolved disagreements.

### *Quality assessment and data extraction*

We evaluated the quality of all included articles according to the rigorous review checklist pro-

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

| Study | Country | Cases | Design | Type of cancer | Stage | Sample site | Methods | Cutoff | Survival analysis | Hazard ratios | Maximum follow-up (months) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Valladares-Ayerbes 2012 | Spain | 52 | R | Gastric | I-IV | Plasma | qRT-PCR | Mean | OS, PFS | Reported | 60 |
| Yu 2014 | China | 157 | R | ESCC | III, IV | Serum | qRT-PCR | Median | OS | Reported | 50 |
| Tanaka 2013 | Japan | 64 | R | ESCC | II-IV | Serum | qRT-PCR | Median | PFS | Reported | 42 |
| Torres 2013 | Poland | 122 | R | EEC | I-IV | Tissues | qRT-PCR | Quartile | OS | Reported | 150 |
| Berghmans 2013 | Belgium | 38 | P | NSCLC | Histology | Tissues | qRT-PCR | Quartile | OS | Reported | 60 |
| Berglund 2013 | Sweden | 61 | R | DLBCL | I-IV | Tissues | qRT-PCR | Mean | OS | SC | 192 |
| Liu 2012 | China | 70 | R | NSCLC | I-IV | Tissues | qRT-PCR | > 2 Fold change | OS | Reported | 30 |
| Yu 2010 | Japan | 99 | R | Pancreatic | I-IV | Tissues | qRT-PCR | 0.64 | OS | Reported | 101 |
| Cao 2014 | China | 100 | R | EOC | I-IV | Tissues | qRT-PCR | Median | OS | Reported | 56 |
| Tang 2013 | China | 126 | R | Gastricr | I-IV | Tissues | qRT-PCR | Scores > 2 | OS/RFS | Reported | 60 |
| Leskela 2011 | Spain | 72 | R | Ovarian | I-IV | Tissues | qRT-PCR | Mean | PFS | Reported | 125 |
| Hamano 2011 | Japan | 98 | R | ESCC | I-IV | Tissues | qRT-PCR | Median | OS | DE | 96.7 |
| Xi 2006 | USA | 24 | R | Colorectal | I-IV | Tissues | qRT-PCR | High: ΔCT > 4.54 | OS | DE | 75 |
| | | | | | | | | Low: ΔCT < 4.54 | | | |
| Karaayvaz 2012 | USA | 34 | R | Endometrial | I-IV | Tissues | qRT-PCR | High: ΔCT > 35.5 | OS | SC | 125 |
| | | | | | | | | Low: ΔCT < 35.5 | | | |
| Toiyama 2014 | Japan | 246 | R | Colorectal | I-IV | Serum | qRT-PCR | Youden Index | OS | Reported | 70 |
| | | | | | | Tissues | | | | | |
| Diaz 2014 | Spain | 122 | R | Colorectal | I-IV | Tissues | qRT-PCR | 0.817 | OS/DFS | SC | 120 |

R: retrospective; P: prospective; NSCLC: non-small-cell lung cancer; ESCC: esophageal squamous cell carcinoma; EEC: endometrioid endometrial cancer; EOC: epithelial ovarian cancer; DLBCL: diffuse large B-cell lymphoma; OS: overall survival; PFS: progression-free survival; RFS: recurrence-free survival: DFS: disease-free survival; DE: data-extrapolated; SC: survival curves; qRT-PCR: quantitative real-time polymerase chain reaction.

**Table 2.** Summary of enrolled studies

| Study | Races | Main histological type | RNA Isolation | Reference genes | Survival analysis | Hazard ratios |
|---|---|---|---|---|---|---|
| Valladares-Ayerbes 2012 | Caucasians | Adenocarcinoma | RNA Kit | 5S rRNA, RNU6B | OS/PFS | 2.24 (1.091, 4.614)) |
| | | | | | | 2.27 (1.093, 4.712 |
| Yu 2014 | Asians | Squamous carcinoma | RNA Kit | Synthetic oligonucleotides | OS | 1.665 (1.135, 2.443) |
| Tanaka 2013 | Asians | Squamous carcinoma | RNA Kit | Synthetic oligonucleotides | PFS | 2.787 (1.1079, 7.9585) |
| | | | Trizol | | | |
| Torres 2013 | Caucasians | --- | RNA Kit | Synthetic oligonucleotides | OS | 2.723 (1.47, 5.043) |
| Berghmans 2013 | Caucasians | Adenocarcinoma | RNA Kit | RNU48/RNU44 | OS | 1.057 (1.232, 1.842) |
| Berglund 2013 | Caucasians | --- | RNA Kit | RNU6B | OS | 2.48 (1.16, 5.3) |
| Liu 2012 | Asians | Squamous, adenocarcinoma | Trizol | RNU6B | OS | 6.02 (1.344, 26.971) |
| | | | RNA Kit | | | |
| Yu 2010 | Asians | Adenocarcinoma | RNA Kit | RNU6B- | OS | 0.454 (0.22, 0.91) |
| Cao 2014 | Asians | Serous carcinoma | RNA Kit | RNU6B | OS | 16.22 (1.27, 33.81) |
| Tang 2013 | Asians | --- | Trizol | U6 | OS/RFS | 0.4 (0.27, 0.82)) |
| | | | | | | 0.51 (0.39, 0.87 |
| Leskela 2011 | Caucasians | Serous carcinoma | RNA kit | 5S rRNA | PFS | 2.24 (1, 5.03) |
| Hamano 2011 | Asians | Squamous,carcinoma | Trizol | RNU48 | OS | 1.74 (1.03, 2.92) |
| Xi 2006 | Caucasians | Adenocarcinoma | Trizol | 5S rRNA | OS | 2.88 (1.26, 6.57) |
| Karaayvaz 2012 | Caucasians | Endometrioid | Trizol | RNU6B | OS | 1.34 (0.47, 3.79) |
| Toiyama 2014 | Asians | --- | RNA Kit | Synthetic oligonucleotides | OS | 2.67 (1.28, 5.67) |
| | | | | | | 0.56 (0.28, 1.1) |
| Diaz 2014 | Caucasians | --- | Trizol | RNU6B | OS/DFS | 0.39 (0.12, 133) |
| | | | | | | 0.59 (0.26, 1.31) |

OS: overall survival; PFS: progression-free survival; RFS: recurrence-free survival; DFS: disease-free survival.

posed by MOOSE. Briefly, the key points of the checklist were as follows: (i) clear statement of the study population, country of origin, and study design; (ii) clear definition of cancer type; (iii) clear definition of outcome assessment; (iv) clear definition of analysis of miR-200c expression; (v) clear statement of cutoff values of miR-200c expression levels; (vi) sufficient period of follow-up. Studies not including these six points were excluded to ensure the quality of the meta-analysis. **Figure 1** shows a flowchart of the process used to identify eligible studies. Three investigators (Zhang, Xi and Cui) extracted the primary information independently, including Cox regression analysis, Kaplan-Meier survival analysis, HR, 95% CI and *p* value. Further information was extracted as follows: first author's surname, publication year, origin of population, disease stage, sample site, method for detecting miR-200c, reference gene, cutoff value, assessment of survival outcome, and follow-up time. If HR and 95% CI values were not reported directly, we used the methods described by Tierney et al. [27] and Parmar et al. [28], to take advantage of other information such as Kaplan-Meier curves to calculate HR and the corresponding 95% CI.

*Statistical analysis*

All statistical analyses were performed using Stata 12.0 software (Stata Corporation, College Station, TX, USA). Forest plots were used to estimate the effect of miR-200c expression on overall survival (OS). HR values > 1 were judged to indicate a close association between high miR-200c expression and poor outcome. Heterogeneity between studies was tested using Cochran Q and $I^2$ statistics. If the $I^2$ statistic, which measures the inconsistency across studies, exceeded 50% or $P < 0.10$, heterogeneity was considered significant, and a random-effect model was used. Otherwise, we employed a fixed-effect model [29]. To determine the source of heterogeneity, we conducted meta-regression and subgroup analyses according to race, cancer type, histological type, and sample site. We conducted a sensitive analysis that investigated the influence of a single study on the overall pooled HR by omitting studies one by one. Publication bias was determined by visually inspecting Begg's funnel plot using Egger's bias indicator test [30]. We considered values statistically significant for $P < 0.05$, except if otherwise specified.

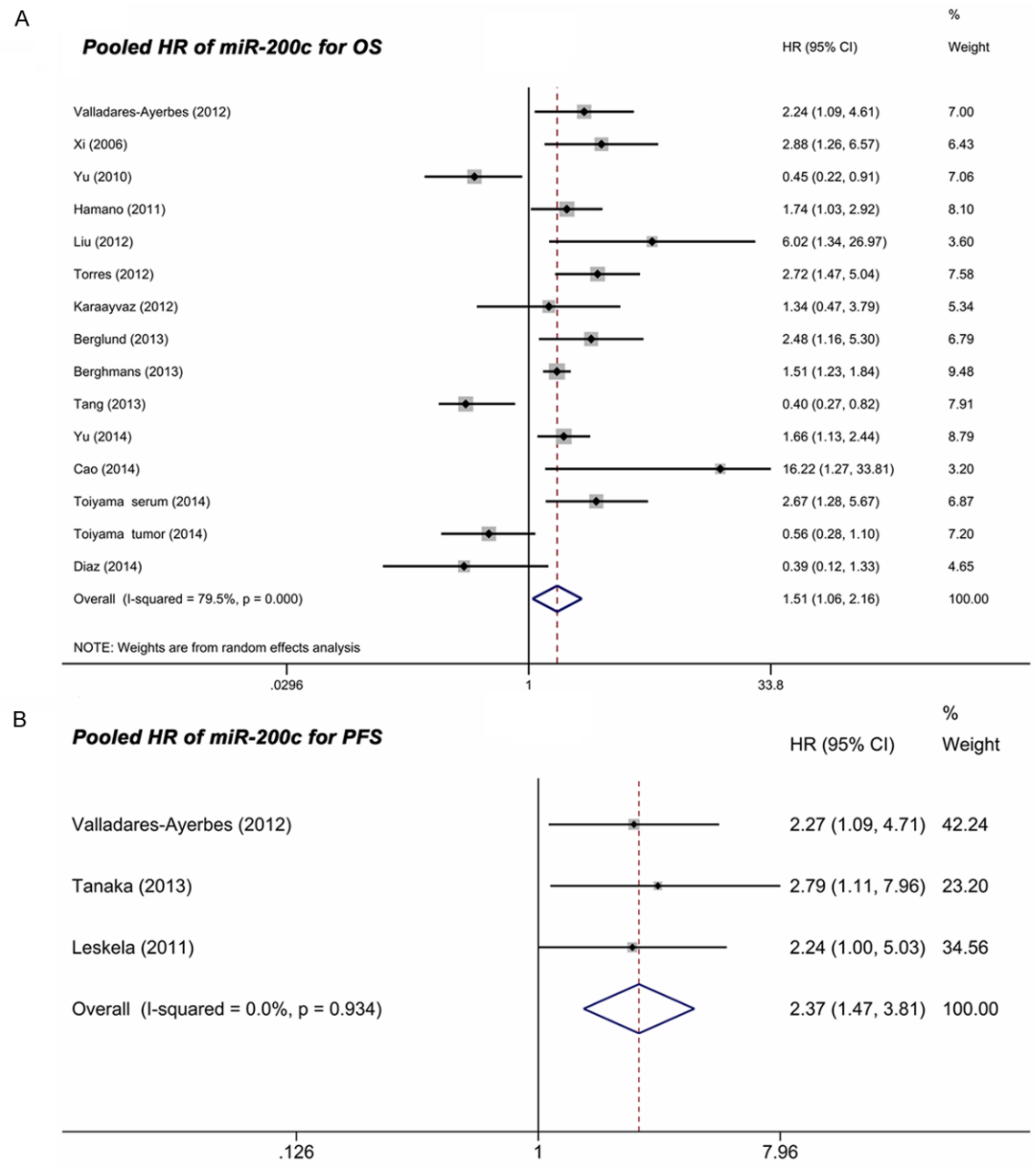


**Figure 2.** Forest plots of OS (A) and PFS (B) in patients with carcinomas.

## Results

After deleting duplicates, 231 records were selected using the search strategy described above, and 180 articles were judged outside the scope of this analysis through skimming the titles and abstracts, because they did not include analyses of miR-200c expression, cancer, prognosis, or did not meet the selection criteria. The 51 remaining studies were further screened using full-text searches, and 35 were excluded as follows: Twenty-eight articles lacked prognostic information or sufficient data calculating for HR. Four articles investigated a set of miRNAs rather than miR-200c alone. One article is a review and two lacked a clear statement of cutoff values. Therefore, the meta-analysis was conducted using 16 studies.

The main characteristics of eligible studies are summarized in **Table 1**. Briefly, we collected data for 1485 participants from Spain [31-33],

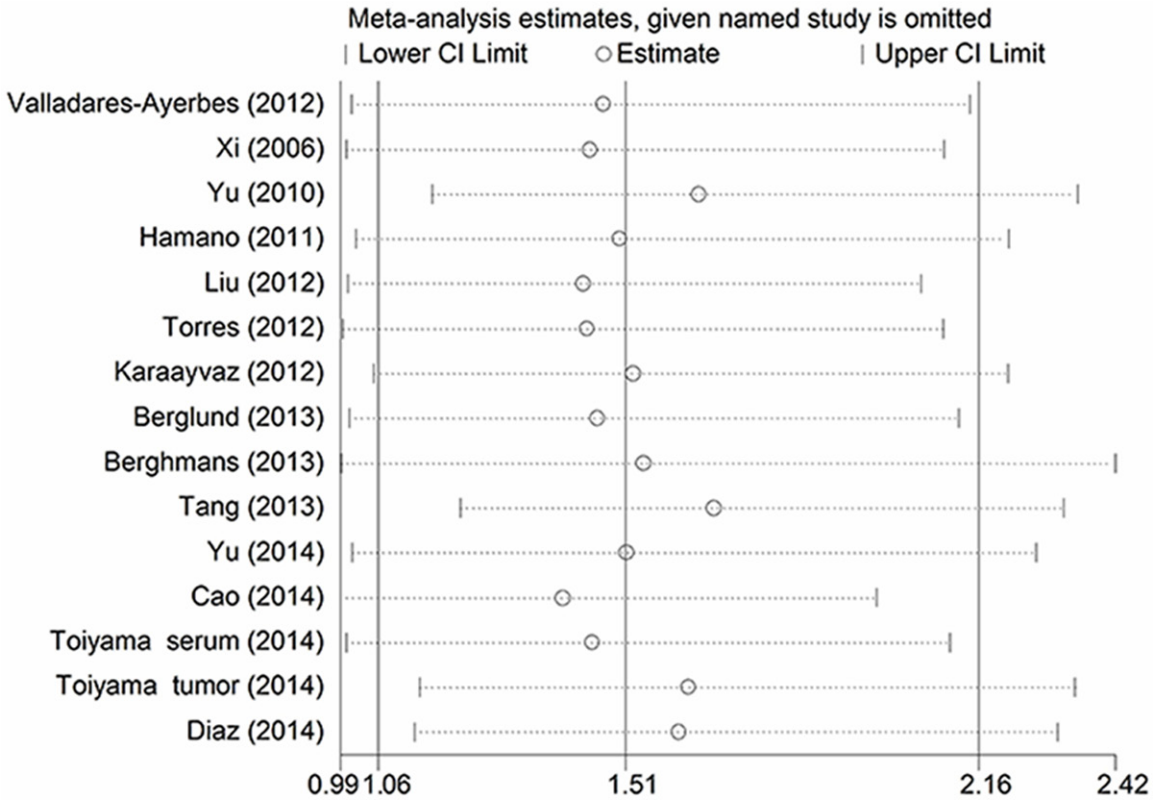


**Figure 3.** The middle vertical line indicates the combined HR, and the two vertical lines represent the corresponding 95% CI values. The middle small circle and two ends of the dotted lines indicates the pooled HR and 95% CI values, respectively, when the study on the left was omitted after each round of analysis.

China [34-37], Poland [38], Sweden [39], Belgium [40], Japan [41-44], and the United States [45, 46]. The studies were retrospective except for one [40] prospective study. Participants were diagnosed with the cancers as follows: lung, gastric, colorectal, esophageal, ovarian, endometrial, pancreatic, and diffuse large B-cell lymphoma. Nine studies [31, 33, 36, 37, 41-44, 46] (n = 924) focused on digestive cancer, two [35, 40] (n = 108) on lung cancer, and three [32, 34, 38] (n = 256) on gynecological tumors. The cancers comprised adenocarcinomas, squamous carcinomas and others. The expression of miR-200c was measured using qRT-PCR, although the RNA extraction methods and reference genes differ among studies. Most studies investigated miR-200c expression in cancer tissues, three [33, 37, 42] focused on expression in peripheral blood, and one [38] measured both. Notably, cutoff values of miR-200c expression differed in each study, including the median applied as well as mean, quartile, 2-fold changes in expression levels. The maximum follow-up period ranges from 30 to 192 months. The detailed HR and corresponding 95% CI values are shown in **Table 2**.

The results of studies that evaluate patients' overall survival are heterogeneous ($P$ = 0.000, $I^2$ = 79.5%). Therefore, when we calculated a pooled HR using a random-effect model, we found that a high level of expression of miR-200c predicted statistically significant poor survival with pooled HR being 1.51 (95% CI 1.06-2.16) (**Figure 2A**). To explore the source of heterogeneity, we performed a sensitive analysis by omitting studies one by one. However, exclusion of any single study did not materially influence the overall combined HR, with a range from 1.40 to 1.67 (**Figure 3**).

Next, we performed meta-regression and subgroup analyses (**Figure 4**). The results indicate that race, cancer type, histological type, and sample site contributed to heterogeneity with p values of 0.658, 0.091, 0.775, and 0.510, respectively (**Table 3**). Further, high levels of miR-200c closely associate with poor OS

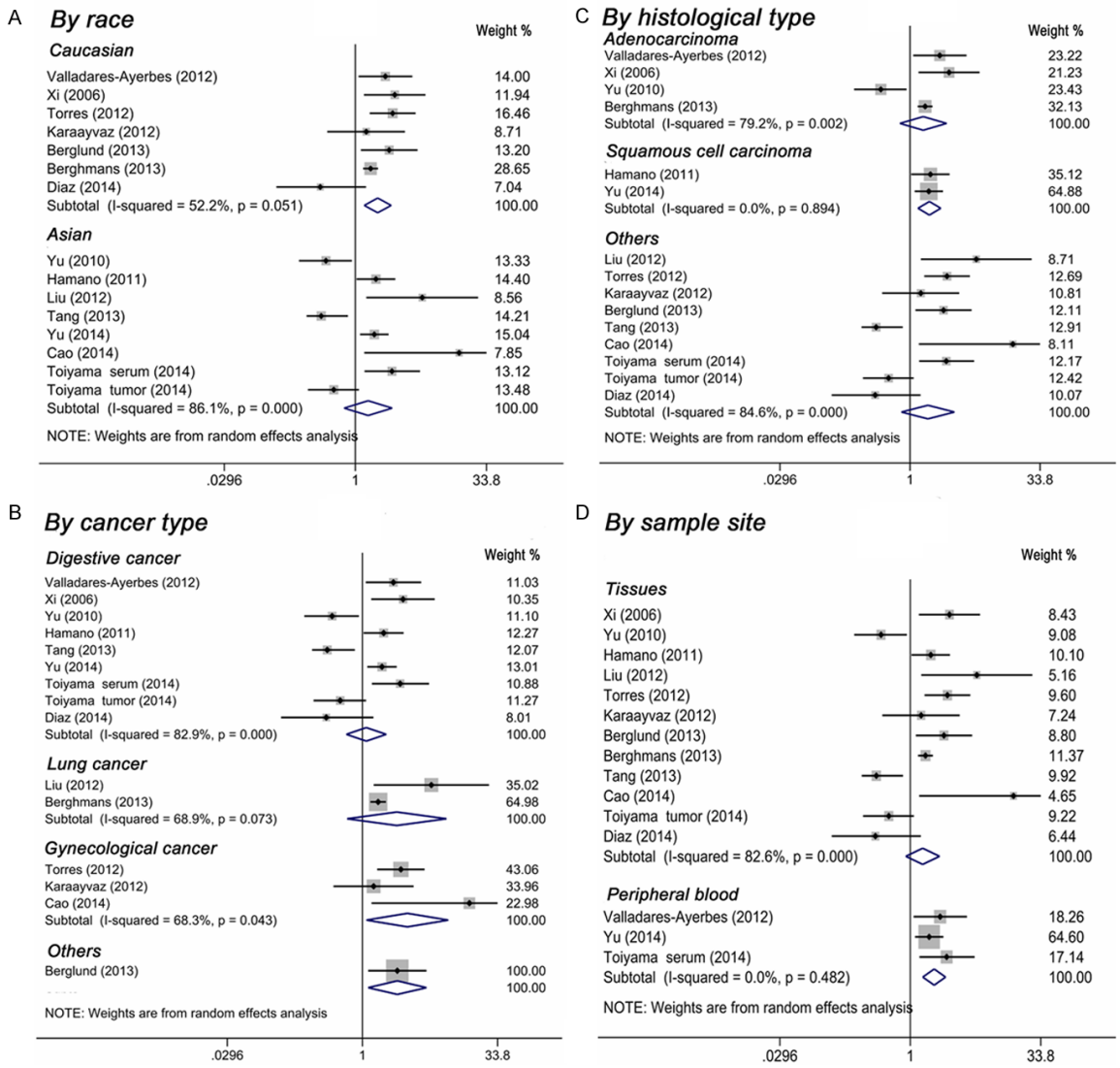


**Figure 4.** Forest plots of pooled HR values for subgroup analysis according to race (A), cancer type (B), histological type (C), and sample site (D).

among Caucasians and gynecological tumors (HR 1.82 (95% CI 1.27-2.26) and 3.23 (95% CI 1.11-9.38). HR was calculated using a random effect model because of the presence of heterogeneity. Interestingly, in studies investigating squamous carcinoma and samples from peripheral blood ($I^2$ = 0.00) that are not heterogeneous, we used a fixed-effect model to calculate a pooled HR and found that high miR-200c expression predicted shorter survival; the combined HR values were 1.69 (95% CI 1.24-2.30) and 1.91 (95% CI 1.40-2.59), respectively. Although the combined HR for Asians was 1.41 (95% CI 0.74-2.96), digestive tumors 1.11 (95% CI 0.66-1.45), lung cancer 2.45 (95% CI 0.67-8.93), and adenocarcinoma 1.43 (95% CI 0.76-2.70), the associations between high miR-200c expression and poor overall survival was not statistically significant.

Three studies discuss progression-free survival (PFS) and are homogeneous ($I^2$ = 0.0%, $P$ = 0.934). Using a fixed-effect model, the pooled HR was 2.37 (95% CI 1.47-3.81) (**Figure 2B**). HR > 2 is generally considered strongly predictive [47]. These results indicate that high levels of miR-200c expression strongly associated with worse PFS in patients with cancer.

Publication bias was evaluated for total OS and PFS using Begg's funnel plots and Egger's tests. The shape of the funnel plots is almost

**Table 3.** Meta-regression and subgroup analysis of the studies reporting the association of high level of miR-200c and overall survival

| Stratified analysis | No. patients | No. studies | Pooled HR (95% CI) | *P* value for meta-regression | $I^2$, % | *P* value for heterogeneity |
|---|---|---|---|---|---|---|
| *Races* | | | | 0.658 | | |
| Caucasians | 453 | 7 | 1.82 (1.27, 2.26) | | 52.2 | 0.051 |
| Asians | 896 | 7 | 1.41 (0.74, 2.69) | | 86.1 | < 0.001 |
| *Cancer type* | | | | 0.091 | | |
| Digestive tumor | 924 | 8 | 1.11 (0.66, 1.45) | | 82.9 | < 0.001 |
| Lung cancer | 108 | 2 | 2.45 (0.67, 8.93) | | 68.9 | 0.073 |
| Gynecological tumor | 256 | 3 | 3.23 (1.11, 9.38) | | 68.3 | 0.043 |
| Others | 61 | 1 | 2.48 (1.16, 5.30) | | --- | --- |
| *Histological type* | | | | 0.775 | | |
| Adenocarcinoma | 213 | 4 | 1.43 (0.76, 2.70) | | 79.2 | 0.002 |
| Squamous carcinoma | 255 | 2 | 1.69 (1.24, 2.30) | | 0.0 | 0.894 |
| Others | 881 | 8 | 1.72 (0.79, 3.34) | | 84.6 | < 0.001 |
| *Sample site* | | | | 0.510 | | |
| Tissues | 1140 | 12 | 1.40 (0.88, 2.21) | | 82.6 | < 0.001 |
| Peripheral blood | 455 | 3 | 1.91 (1.40, 2.59) | | 0.0 | 0.482 |

symmetrical (**Figure 5**). Egger's tests provided statistical evidence for symmetry. As we expected, the *p* values of Egger's tests of OS and PFS were 0.768 and 0.262, respectively, indicating that the present study was not subject to publication bias.

## Discussion

To the best of our knowledge, the present study is the first comprehensive meta-analysis of 16 studies designed to evaluate the potential of high expression levels of miR-200c to predict the survival of patients with different cancers. Here, we combined the outcomes from seven countries of 1485 patients with cancer and found that overexpression of miR-200c predicted significantly worse OS (HR 1.51; 95% CI 1.06-2.16). Moreover, overexpression of miR-200c strongly associated with shorter PFS in patients with cancer (HR 2.37; 95% CI 1.47-3.81). Subgroup analysis revealed that high levels of miR-200c associated with poor survival of Caucasians, patients with gynecological tumors, and patients with squamous cell carcinoma. Further detection of overexpression of miR-200c in peripheral blood predicted poor outcome in patients with cancer.

However, there are some limitations of this meta-analysis. First, because of the presence of the heterogeneity across studies, we used a random-effect model to combine the data. In conducting a sensitive analysis, deletion of a single study did not indicate the source of heterogeneity. Meta-regression and subgroup analyses showed that races, cancer types, histological types, and sample sites accounted for heterogeneity to some degree, because the characteristics of patients with cancer and genetic alterations among various carcinomas might differ. We did not detect heterogeneity among the squamous cell carcinoma ($I^2$ = 0.0, *P* = 0.894) and peripheral blood subgroups ($I^2$ = 0.0, *P* = 0.482). The inconsistency among RNA isolation methods and reference genes might generate a certain degree of heterogeneity. Second, these conclusions are weakened considering the different definitions of the miR-200c cutoff values. The approach using a quantitative combination of HR values from different studies cannot determine the value of "high". Third, caution should be exercised to interpret the results for PFS. Because only three studies assess PFS, the insufficient data may introduce bias that compromised the validity of our publication bias tests. Therefore, large-scale studies are required to define the association between miR-200c expression and PFS. Fourth, are tissues or peripheral blood the most appropriate sources for analyzing miR-200c expression? Most research focuses on

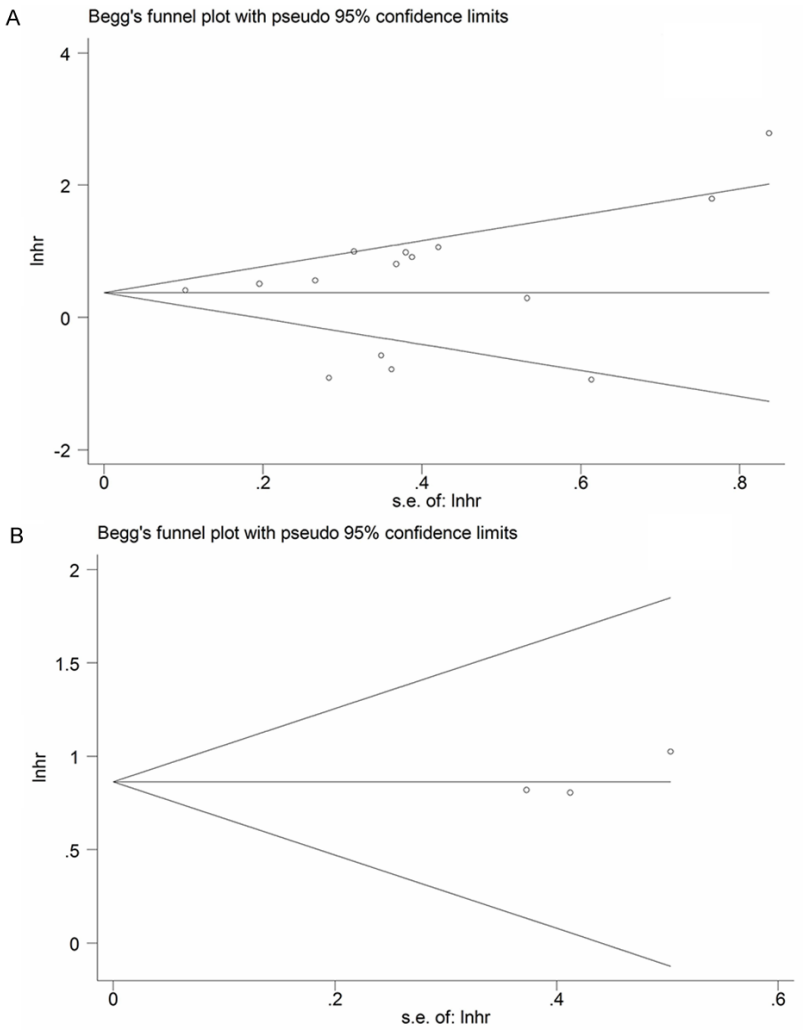


**Figure 5.** Begg's funnel plot to evaluate OS (A) and PFS (B).

tissues expression. However, analysis of miR-200c in blood is less invasive than biopsies and allows continuous monitoring of a patient's condition. Mitchell et al. [48] demonstrated that miRNAs present in blood are stable after being subjected to severe conditions that would normally degrade most miRNAs. Despite the presence of RNase activity in blood, incubation of blood at room temperature for up to 24 h, or after up to eight freeze-thaw cycles, has little effect on miRNAs levels. Moreover, commercially available qPCR kits accurately detect circulating miRNAs [49]. Therefore, this promising field deserves more attention.

Intensive investigations have focused on miRNAs because they were discovered in 1993 [50, 51], and we summarize the evidence of the potential role of miR-21 in colorectal carcinogenesis in a previous study [11]. Based on analysis of transcriptomedata,wefocused our attention on miR-200c expression, which is associated with potential cancer-risk regions along with miR-21 [12]. MiR-200c is overexpressed or downregulated exclusively in certain types of cancer. The stability of miRNAs and their ease of detection in a vast range of specimens such as fresh tissues, formalin-fixed paraffin-embedded tissues, plasma, and serum as well as other body fluids, indicate that miRNAs may serve as biomarkers for predicting cancer prognosis. For example, Toiyama et al. [43] and Diaz et al. [31] found that high levels of miR-200c predict worse survival. In contrast, Diaz et al. [31] reported that miR-200c overexpression predicts longer survival of patients with cancer. We settled this inconsistency with our meta-analysis and calculated a pooled HR = 1.51, indicating that upregulated expression of miR-200c may predict poor outcome.

The strengths of our meta-analysis are as follows: First, we performed the meta-analysis in strict compliance with the MOOSE guidelines and carefully and systematically retrieved relevant publications from PubMed and Embase. Second, we rigorously selected eligible studies according to our detailed selection and exclusion criteria. Third, sensitive analysis, meta-regression, and subgroup analysis were employed to evaluate or minimize heterogeneity across studies. Fourth, the results of Begg's funnel plots and Egger's tests indicated minimal publication bias.

We suggest that researchers consider other aspects of analyzing miRNA expression as bio-

markers for disease. First, although the levels of miRNAs in blood are used for diagnosis [52-54], investigation on their prognostic role is in its infancy. The simplicity of collecting blood samples together with the stability of circulating miRNAs raises the possibility of using miRNAs to monitor tumor dynamics after curative resection. Second, unlike in tumor tissues, RNU6B may not serve as a reliable reference gene, because its levels in peripheral blood vary under different conditions. Typically, quantitating the levels of circulating miRNAs requires a "spike in" [55] such as cel-miR-39 used by Yu et al. [37], Tanaka et al. [42], and Toiyama et al. [43], to serve as an internal standard for normalizing qRT-PCR data. Investigators have identified miR-16 as reference gene for normalizing the levels of circulating miRNAs [56]. However, miR-16 may be involved in tumorigenesis [57, 58]. Therefore, an optimal reference gene (or genes, depending on the source) must be established. Third, a standard cutoff value must be defined to allow valid comparisons among studies.

Our meta-analysis shows that levels of expression of miR-200c serve as a suitable prognostic marker for patients with cancer. Further, high levels of expression of miR-200c predict worse survival of Caucasians and patients with gynecological tumors and squamous cell carcinomas. Moreover, detection of miR-200c indicates poor prognosis, and upregulated expression of miR-200c indicates shorter PFS. Because the number of relevant studies is insufficient to validate these findings, further investigations are required.

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## Disclosure of conflict of interest

None.

**Address correspondence to:** Dr. Lin Chen, Department of General Surgery, Chinese People's Liberation Army General Hospital, 28 Fuxing Road, Beijing 100853, China. Tel: +86-10-66938128; Fax: +86-10-68181689; E-mail: chenlinbj@sina.com

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