RESEARCH ARTICLE

# How to Explain the Contradiction of microRNA 200c Expression and Survival in Solid Tumors?: a Meta-analysis

**Hui-Yu Wang[1], Jie Shen[2], Chun-Ping Jiang[2], Bao-Rui Liu[1,2]***

## Abstract

**MicroRNA 200c is a microRNA 200 family member that plays an important role in regulation of the epithelial-to-mesenchymal transition (EMT). The prognostic value of microRNA 200c in solid tumors remains controversial because of inconsistent data. Here, we report a meta-analysis of the association of microRNA 200c expression and survival in patients with solid tumors. Pubmed was searched up to November 2013 for studies investigating microRNA 200c expression and overall survival (OS) in solid tumors. Hazard ratios (HRs) with 95% confidence intervals (CIs) for OS were extracted from each study. Pooled HR and CIs were calculated using the Mantel-Haenszel fixed-effects models. A total of five studies evaluating colorectal cancer, gastric cancer, ovarian cancer, pancreatic cancer and endometrial cancer were included in the analysis. Data were divided into tissue microRNA 200c expression group and serum microRNA 200c expression group. The combined HRs [95%CIs] estimated for OS were 0.62 [0.42-0.91] and 2.16 [1.32-3.52] respectively. Low expression of microRNA 200c in tumor tissue and high expression of microRNA 200c in serum are associated with worse survival in solid tumors. Further study is needed to elucidate this contradiction.**

**Keywords:** microRNA 200c expression - solid tumors - survival - meta-analysis

*Asian Pac J Cancer Prev,* **15 (8)**, 3687-3690

## Introduction

MicroRNAs (miRNAs) are 21-23 nucleotides long non-coding RNA molecules that regulate various biological processes by modulating the expression of their targeted mRNAs (Rana, 2007; Nilsen, 2007; Filipowicz et al., 2008). Downregulation of global miRNAs can be found in tumors compared with normal tissues by miRNA expression profiling analysis which reveal the potential corelation of miRNAs and tumorigenesis (Lu et al., 2005; Thomsom et al., 2006; Xu et al., 2013).

MiRNA 200 family consists of five miRNAs including miRNA-200a, -200b, -200c, -141 and -429 and therein miRNA 200c is widely investigated in recent years. It is documented that the suppression of miRNA200c by interleukin 6 (IL6) drives transformation and tumorigenesis (Rokavec et al., 2012). Increasing evidence also demonstrate that miRNA 200c plays an critical role in epithelial-mesenchymal transition (EMT) which enhance the migration and invasion of tumor cells and eventually lead to metastasis (Mongroo et al., 2010; Dykxhoorn 2010). Moreover, restoration of miRNA 200c to certain solid tumors showed the ability to increase the sensitivity to chemo drug or radiation by various mechanisms (Cochrane et al., 2009; Cittelly et al., 2012; Kopp et al., 2012; Chang et al., 2013; Shi et al., 2013).

However, the prognostic relevance of miRNA 200c expression in solid tumors remains controversial. Varied survival outcomes can be found in studies focused on the prognostic value of miRNA 200c expression. Therefore, we conducted the first comprehensive meta-analysis of published literature on this topic to summarize the evidence.

## Materials and Methods

*Search strategy*

Pubmed was searched in November 2013. The following keywords were combined: "microRNA 200c" and "cancer". No language and time restrictions were made.

*Inclusion criteria*

In order to be eligible, studies had to: (i) discuss the relevance of microRNA 200c expression and OS, (ii) provide sufficient data for extracting or estimating HR and its 95% CI.

*Exclusion criteria*

Studies were excluded from the analysis if: (i) the articles were not written in English, (ii) the articles were review or letters, (iii) studies did not investigate solid tumors , (iv) primary data for meta-analysis could not be extracted or calculated as lacking essential information.

*[1]Nanjing Drum Tower Hospital, Clinical Cellege of Nanjing Medical University [2]The Comprehensive Cancer Center of Drum Tower Hospital, Medical School of Nanjing University, Nanjing, China *For correspondence: baoruiliu@nju.edu.cn*

**Table 1. Baseline Characteristics of Included Studies**

| First author (year) | Tumor type | No. of patients | Sample size | Detection methods | High expression, % | HR estimation |
|---|---|---|---|---|---|---|
| Manuel Valladares-Aye-rbes (2012) | gastric cancer | 52 | serum | qRT-PCR | 53.8% | Data extrapolated |
| Mihriban Karaayvaz (2012) | endometrial cancer | 34 | FFPE tissue | qRT-PCR | 50.0% | Data extrapolated |
| Jun Yu (2010) | pancreatic cancer | 99 | FFPE tissue | qRT-PCR | 21.2% | Data extrapolated |
| Sergio Marchini (2011) | ovarian cancer | 89 | frozen tissue | qRT-PCR | 65.2% | Reported in text |
| Yuji Toiyama (2013) | colorectal cancer | 182/156 | serum/FFPE tissue | qRT-PCR | 50%/50% | Reported in text |

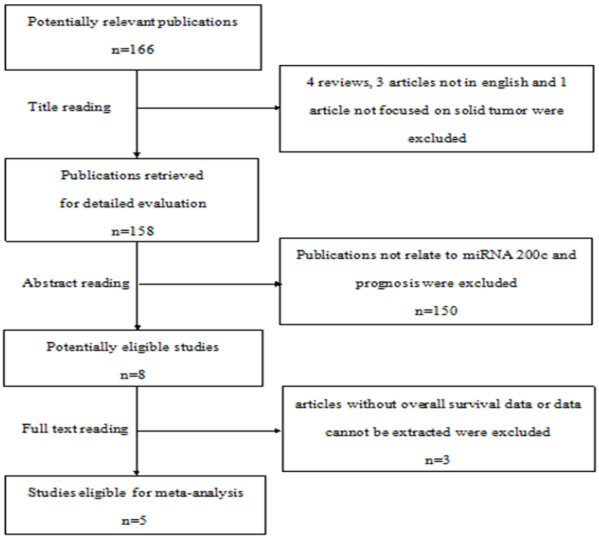


**Figure 1. Flowchart of the Selection**

*Data extraction*

The primary data were hazard ratio (HR) and its 95% confidence interval (CI) of OS. Three reviewers (HY Wang, J Shen, CP Jiang) independently extracted the primary data and baseline characteristics of included studies. Only the Kaplan-Meier survival curves but not HR and its 95% CI were given in some included articles. As for these articles, methods according to the work of Parmar, Williamson and Tierney were used to calculate HR (Parmar et al., 1998; Williamson et al., 2002; Tierney et al., 2007). The baseline characteristics included first author, publication year, tumor type, study size, sample site, methods to detect microRNA 200c expression, percentage of patients with high miRNA 200c expression and HR estimation methods. Disagreements were resolved by discussion.

*Statistical methods*

LogHR and standard error (SE) were calculated by using the software designed by Matthew Sydes and Jayne Tierney (Medical Research Council Clinical Trials Unit, London, UK) (Tierney et al., 2007). The pooled HR was gained using fixed or random-effects models according to the heterogeneity between studies. Heterogeneity was evaluated with the Cochran's Q test as well as the $I^2$ index and was defined as $p < 0.10$ or $I^2 > 50\%$ (Higgins et al., 2003). Publication bias was evaluated by Begg's funnel plots and Egger's test.

Forrest plots showed the pooled HR and HR>1 indicated worse survival outcome. All calculations were conducted by use of Review Manager Version 5.2 (The Cochrane Collaboration, Software Update, Oxford, UK) or Stata software (Stata Corporation, College Station, Texas).

**Table 2. Summary HRs for the Association of the miRNA 200c Expression with Overall Survival**

| Groups | Number of studies | Pooled HR [95%CI] | *p* value | $I^2$ value |
|---|---|---|---|---|
| serum miRNA 200c expression | 2 | 2.16 [1.32-3.52] | 0.002 | 0% |
| tissue miRNA 200c expression | 4 | 0.62 [0.42-0.91] | 0.01 | 5% |

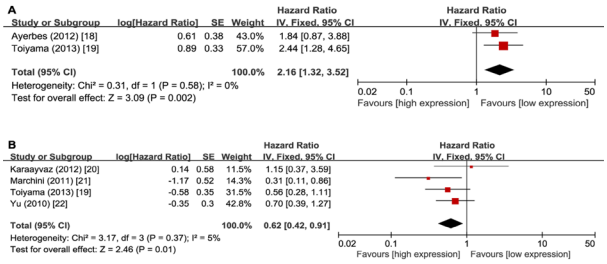


**Figure 2. Forrest Plots of Estimated Hazard Ratios (HRs) for (A) Serum miRNA200c Expression and OS, and (B) Tumor Tissue miRNA200c Expression and OS**

## Results

*Characteristics of eligible studies*

A total of 166 articles were yielded after the literature search. After title reading, abstract reading and full-text reviewing, five studies were included (Figure 1). Eligible studies investigated colorectal cancer, gastric cancer, ovarian cancer, pancreatic cancer and endometrial cancer. The sample size ranged from 34 to 182 patients. The included studies were conducted between 2010 and 2013. The main features of these studies were listed in Table 1.

*Serum miRNA 200c expression and survival*

Two of the five included studies evaluated the association of serum miRNA 200c expression and OS (Valladares-Ayerbes et al., 2012; Toiyama et al., 2013). HR and its 95% CI were given in one study enrolling colorectal cancer patients (Toiyama et al., 2013). In the other study focused on gastric cancer patients, HR and its 95% CI were extracted from the given Kaplan-Meier survival curve (Tierney et al., 2007; Valladares-Ayerbes et al., 2012). Fixed-effects model was used and the pooled HR (high vs low) was 2.16 (95%CI: 1.32-3.52) ($I^2$=0%, $p$=0.002) (Figure 2A, Table 2). The result showed an increased mortality in patients with high expression level of miRNA 200c in serum.

*Tissue miRNA 200c expression and survival.*

Yuji Toiyama's study also analysed the corelation of OS and miRNA 200c expression in FFPE tissue (Toiyama et al., 2013). Like this article, other three studies estimated the prognostic value of miRNA 200c expression in tumor tissue (Yu et al., 2010; Marchini et al., 2011; Karaayvaz et al., 2012). Among these studies, two studies reported

HR and its 95% CI (Marchini et al., 2011; Toiyama et al., 2013) and the rest ones were processed by Matthew Sydes and Jayne Tierney's method to acquire survival data (Tierney et al., 2007; Marchini et al., 2011; Karaayvaz et al., 2012). HRs (high vs low) were pooled by fixed-effects model (Figure 2B, Table 2). Opposite to the serum miRNA 200c expression, the result (pooled HR [95%CI]: 0.62 [0.42-0.91], $I^2$=5%, $p$=0.01 ) demonstrated that low expression level of miRNA 200c in tumor tissue indicated worse survival.

*Publication bias*

We used Begg's funnel plots and Egger's test to evaluate the publication bias of the included studies. As shown in figure 3, the funnel plots were almost symmetric. The $p$ value of Egger's regression intercepts in the meta-analysis of tissue microRNA 200c was 0.731. Therefore, there was no significant publication bias exists in our meta-analysis.

## Discussion

In miRNA 200 family, miRNA 200c was one of the most studied members and showed great potential in cancer therapy. Matjaz Rokavec reported that the supression of miRNA 200c by IL6 activated a feed-forward inflammatory signaling circuit which drives transformation and tumorigenesis of normal cells and loss of IL6 and conservation of miRNA 200c impairs tumorigenesis significantly (Rokavec et al., 2012).

Together with the involvement of tumorigenesis, the corelation of miRNA 200c and EMT was discovered. It is well known that EMT of cancer cells diminishes the epithelial characteristics such as supression of E-cadherin, cytokeratin, occludin and desmoplakin. Meanwhile, EMT increases mesenchymal attributes like expression of vimentin, matrix metalloproteinase family members and E-cadherin transcriptional repressors ZEB1 and ZEB2 (Bonnomet et al., 2010). These characteristics endow cancer cells with enhanced ability of migration and invasion and trigger the cascade of metastasis. Moreover, the evidence of molecular links between EMT and stemness of cancer cells further suggested that interventions to EMT progress may provide new thrapeutic strategies for cancer (Scheel et al., 2012).

It was reported that miRNA 200c targets ZEB1 and ZEB2 (Hurteau et al., 2007; Cochrane et al., 2009). Reintroduction of miRNA 200c restored the expression of E-cadherin and significantly inhibited the migration and invasion in cancer cell line researches (Cochrane et al., 2009). In addition, Florian Kopp reported that miRNA 200c targeted TrkB and Bmi1 so that increasing the sensitivity of breast cancer cells to doxorubicin (Kopp et al., 2012). Another study demonstrated that RhoE was also a target of miRNA 200c and the sensitivity to DDP could be improved by miRNA 200c (Chang et al., 2013). Besides, it was documented that miR-200c targeted class III b-tubulin gene (TUBB3), increasing sensitivity to taxanes in vitro (Cittelly et al., 2012). Radiosensitivity of a non-small cell lung cancer cell line A549 was also increased by applying miRNA 200c (Shi et al., 2013). These results indicated a protective role of miRNA 200c in solid tumors. Our meta-analysis showed the pooled HR (high vs low) for tissue miRNA 200c and OS is 0.62 [0.42-0.91] (HR [95%CI]). This result is in accordance with the verified protective efficacy of miRNA 200c in cancer.

However, contradiction emerged when it came to miRNA 200c expression in serum. Our result of meta-analysis for serum miRNA 200c expression and OS manifested that high expression level in serum correlated with worse survival. The pooled HR and its 95%CI were 2.16 [1.32-3.52]. How to explain this inconformity? Firstly, because of the small sample size, this inconformity could be occasional. Further studies focusing on this topic are necessary to validate it. Even so, it is still valuable to discuss the potential reason of this inconformity. In one of our included studies, Yuji Toiyama studied both serum and tissue miRNA 200c expression (Toiyama et al., 2013). Their results demonstrated that only serum miRNA 200c expression possess the ability to predict OS (HR [95%CI]: 2.43 [1.26-4.68]), but the result of miRNA 200c expression in tumor tissue was not significant (HR [95%CI]: 0.56 [0.28-1.10]). Yuji Toiyama also found that, compared with the primary tumor, the matched liver metstases had higher expression level of miRNA 200c. Hence, the origin of serum miRNA 200c was conjectured to be the metastatic site. On the other side, we put a hypothesis forward that miRNA 200c in serum may be a mirror of circulating tumor cells (CTCs). Chang and colleagues using model systems showed that around $1\times10^6$ tumor cells per gram tumor tissue could be introduced daily into the blood stream (Chang et al., 2000). Though 85% CTCs become broken and disappear within 5 minutes, they still could be vital contributors of serum miRNA 200c (Berezovskaya et al., 2005). In other words, higher level of miRNA 200c may represent more tumor cells entering the circulation. It was documented that CTCs predict worse survival in various cancers (Mocellin et al., 2006; Rahbari et al., 2010; Wang et al., 2011; Ma et al., 2012; Zhang et al., 2012). The result of our meta-analysis could be well explained if the origin of serum miRNA 200c is CTCs. Though these were speculative, we believed the correlation of serum miRNA 200c, metastasis and CTCs may be a key to figure out the inconformity. Besides, the possibility of the increased active secretion of miRNA 200c needs to be discussed. Kosaka and colleagues proved that the secretion of miRNAs was regulated by a ceramide-dependent pathway (Kosaka et al., 2010). The dysfunction of the regulation pathway may also lead to the higher expression level of serum miRNA 200c, while no related studies could be found to date. Eagerly, further studies are needed to prove the hypotheses and ravel out the puzzle.

Limitations of the present meta-analysis need to be discussed. Firstly, HRs and 95%CI of some included studies were extracted. Log (HR) and se (log (HR)) were then calculated by the software provided by Matthew Sydes and Jayne Tierney. Potential biases may relate to this process. Secondly, only five studies fit the included criteria and they investigated five different cancers. More studies in future could be added into our meta-analysis to validate the present results and subgroup analysis should be conducted according to the tumor type. At last,

our meta-analysis only used published data. Updated individual patient data were not obtained. If those data were added to our analysis, the accuracy and determinacy could be better.

In conclusion, our meta-analysis suggest that both serum and tissue miRNA 200c expression could predict OS in solid tumors. Low expression of miRNA 200c in tumor tissue predict worse OS while low expression of miRNA 200c in serum are associated with better survival in solid tumors. To figure out the reason of the opposite results, more studies especially those focused on the origin of serum miRNA 200c are needed in future.

## References


Berezovskaya O, Schimmer AD, Glinskii AB, et al (2005). Increased expression of apoptosis inhibitor protein XIAP contributes to anoikis resistance of circulating human prostate cancer metastasis precursor cells. *Cancer Res*, **65**, 2378-86.

Bonnomet A, Brysse A, Tachsidis A, et al (2010). Epithelial-to-mesenchymal transitions and circulating tumor cells. *J Mammary Gland Biol Neoplasia*, **15**, 261-73.

Chang L, Guo F, Wang Y, et al (2013). MicroRNA-200c Regulates the Sensitivity of Chemotherapy of Gastric Cancer SGC7901/DDP Cells by Directly Targeting RhoE. *Pathol Oncol Res*, **20**, 93-8.

Chang YS, Tomaso E, McDonald DM, et al (2000). Mosaic blood vessels in tumors: frequency of cancer cells in contact with flowing blood. *Proc Natl Acad Sci USA*, **97**, 14608-13.

Cittelly DM, Dimitrova I, Howe EN, et al (2012). Restoration of miR-200c to ovarian cancer reduces tumor burden and increases sensitivity to paclitaxel. *Mol Cancer Ther*, **11**, 2556-65.

Cochrane DR, Spoelstra NS, Howe EN, et al (2009). MicroRNA-200c mitigates invasiveness and restores sensitivity to microtubule-targeting chemotherapeutic agents. *Mol Cancer Ther*, **8**, 1055-66.

Dykxhoorn DM (2010). MicroRNAs and metastasis: little RNAs go a long way. *Cancer Res*, **70**, 6401-6.

Filipowicz W, Bhattacharyya SN, Sonenberg N (2008). Mechanisms of post-transcriptional regulation by microRNAs: are the answers in sight? *Nat Rev Genet*, 9, 102-14.

Higgins JP, Thompson SG, Deeks JJ, et al (2003). Measuring inconsistency in meta-analyses. *BMJ*, **327**, 557-60.

Hurteau GJ, Carlson JA, Spivack SD, et al (2007). Overexpression of the microRNA hsa-miR-200c leads to reduced expression of transcription factor 8 and increased expression of E-cadherin. *Cancer Res*, **67**, 7972-6.

Karaayvaz M, Zhang C, Liang S, et al (2012). Prognostic significance of miR-205 in endometrial cancer. *PLoS One*, **7**, e35158.

Kopp F, Oak PS, Wagner E, et al (2012). miR-200c sensitizes breast cancer cells to doxorubicin treatment by decreasing TrkB and Bmi1 expression. *PLoS One*, **7**, e50469.

Kosaka N, Iguchi H, Yoshioka Y, et al (2010). Secretory mechanisms and intercellular transfer of microRNAs in living cells. *J Biol Chem*, **285**, 17442-52.

Lu J, Getz G, Miska EA, et al (2005). MicroRNA expression profiles classify human cancers. *Nature*, **435**, 834-8.

Ma XL, Xiao ZL, Liu L, et al (2012). Meta-analysis of circulating tumor cells as a prognostic marker in lung cancer. *Asian Pac J Cancer Prev*, **13**, 1137-44.

Marchini S, Cavalieri D, Fruscio R, et al (2011). Association between miR-200c and the survival of patients with stage I epithelial ovarian cancer: a retrospective study of two independent tumour tissue collections. *Lancet Oncol*, **12**, 273-85.

Mocellin S, Hoon D, Ambrosi A, et al (2006). The prognostic value of circulating tumor cells in patients with melanoma: a systematic review and meta-analysis. *Clin Cancer Res*, **12**, 4605-13.

Mongroo PS, Rustgi AK (2010). The role of the miR-200 family in epithelial-mesenchymal transition. *Cancer Biol Ther*, **10**, 219-22.

Nilsen TW (2007). Mechanisms of microRNA-mediated gene regulation in animal cells. *Trends Genet*, **23**, 243-9.

Parmar MK, Torri V, Stewart L, (1998). Extracting summary statistics to perform meta-analyses of the published literature for survival endpoints. *Stat Med*, **17**, 2815-34.

Rahbari NN, Aigner M, Thorlund K, et al (2010). Meta-analysis shows that detection of circulating tumor cells indicates poor prognosis in patients with colorectal cancer. *Gastroenterology*, **138**, 1714-26.

Rana TM (2007). Illuminating the silence: understanding the structure and function of small RNAs. *Nat Rev Mol Cell Biol*, **8**, 23-36.

Rokavec M, Wu W, Luo JL, et al (2012). IL6-mediated suppression of miR-200c directs constitutive activation of inflammatory signaling circuit driving transformation and tumorigenesis. *Mol Cell*, **45**, 777-89.

Scheel C, Weinberg RA (2012). Cancer stem cells and epithelial-mesenchymal transition: concepts and molecular links. *Semin Cancer Biol*, **22**, 396-403.

Shi L, Zhang S, Wu H, et al (2013). MiR-200c Increases the Radiosensitivity of Non-Small-Cell Lung Cancer Cell Line A549 by Targeting VEGF-VEGFR2 Pathway. *PLoS One*, **8**, e78344.

Thomson JM, Newman M, Parker JS, et al (2006). Extensive post-transcriptional regulation of microRNAs and its implications for cancer. *Genes Dev*, **20**, 2202-7.

Tierney JF, Stewart LA, Ghersi D, et al (2007). Practical methods for incorporating summary time-to-event data into meta-analysis. *Trials*, **8**, 16.

Toiyama Y, Hur K, Tanaka K, et al (2013). Serum miR-200c Is a Novel Prognostic and Metastasis-Predictive Biomarker in Patients With Colorectal Cancer. *Ann Surg*, doi: 10.1097/SLA.0b013e3182a6909d.

Valladares-Ayerbes M, Reboredo M, Medina-Villaamil V, et al (2012). Circulating miR-200c as a diagnostic and prognostic biomarker for gastric cancer. *J Transl Med*, **10**, 186.

Wang FB, Yang XQ, Yang S, et al (2011). A higher number of circulating tumor cells (CTC) in peripheral blood indicates poor prognosis in prostate cancer patients--a meta-analysis. *Asian Pac J Cancer Prev*, **12**, 2629-35.

Williamson PR, Smith CT, Hutton JL, et al (2002). Aggregate data meta-analysis with time-to-event outcomes. *Stat Med*, **21**, 3337-51.

Xu TP, Zhu CH, Zhang J, et al (2013). MicroRNA-155 expression has prognostic value in patients with non-small cell lung cancer and digestive system carcinomas. *Asian Pac J Cancer Prev*, **14**, 7085-90.

Yu J, Ohuchida K, Mizumoto K, et al (2010). MicroRNA, hsa-miR-200c, is an independent prognostic factor in pancreatic cancer and its upregulation inhibits pancreatic cancer invasion but increases cell proliferation. *Mol Cancer*, **9**, 169.

Zhang L, Riethdorf S, Wu G, et al (2012). Meta-analysis of the prognostic value of circulating tumor cells in breast cancer. *Clin Cancer Res*, **18**, 5701-10.