Original Article
Prognostic role of microRNA-210 in various carcinomas: a meta-analysis

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Abstract: Background: MicroRNA (miRNA) expressive alterations are associated with cancer and have potential diagnostic and prognostic values in various malignancies. Here, we summarize the global predictive role of miR-210 expression for survival in patients with a variety of carcinomas. Methods: Eligible studies were identified through multiple search strategies. Data were assembled from studies investigating the relationship between miR-210 expression and survival in cancer patients. Hazard ratio (HR) was used as the common measure of association across studies: relative risk (RR) was considered equivalent to HR. Combined hazard ratios (HRs) of miR-210 for outcome were analyzed. Results: A total of 10 studies dealing with various carcinomas were included for this global meta-analysis. For overall survival (OS), the pooled hazard ratio (HR) of higher miR-210 expression in cancerous tissue was 2.41 (95% CI: 1.31-4.44), which could significantly predict poorer survival in general carcinomas. For distant-free, relapse-free or progressive-free survival, elevated miR-210 was also a significant predictor, with a pooled HR of 2.84 (95% CI: 2.10-3.83). Importantly, subgroup analysis suggested that higher expression of miR-210 correlated with worse OS in breast cancer: HR 4.34, 95% CI: 1.63-11.55. Conclusions: Our findings reveal that miR-210 detection has a prognostic value in patients with cancer, especially in breast cancer.

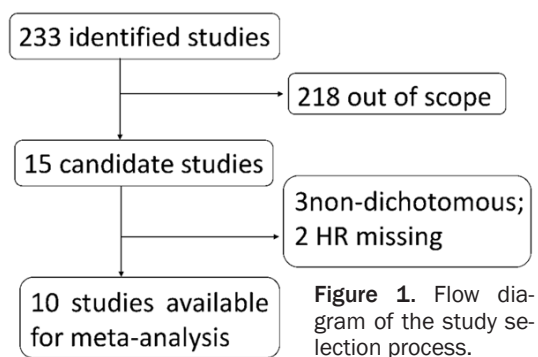Keywords: miR-210, cancer, prognosis, meta-analysis

Introduction

MicroRNAs are evolutionary conserved, small non-coding molecules with approximately 19-24 nucleotides in length, which have been shown to regulate multiple biological processes such as cell proliferation, cell differentiation, cell apoptosis and cell cycle regulation [1]. They are also involved in tumorigenic procedures, including inflammation, adhesion, migration, angiogenesis, invasion, and apoptosis. Expression levels of miRNA have been systematically analyzed using tissue and serum samples [2, 3]. Notably, it has been identified that a specific set of miRNAs are upregulated by hypoxia [4]. Among uncovered hypoxia-regulated miRNAs, miR-210 is the most ubiquitously upregulated one in hypoxic tissues [4-6].

In clinical studies, Mir-210 has been reported to be elevated in several types of cancers, including breast cancer [7-10], pediatric osteosarcoma [11], soft-tissue sarcoma [12], non-small cell lung cancer [13, 14], colorectal cancer [15], clear cell renal cancer [16, 17], glioma [18, 19], pancreatic ductal adenocarcinomas [20], head and neck squamous cell carcinoma [21]. Recently, some studies found that a higher expression of miR-210 was significantly associated with worse outcome [8, 11, 12, 19, 22]. However, other studies presented insignificant or inverse results [16, 17]. Due to the inconsistency of the existing literature, we conducted a systematic review and meta-analysis to summarize the findings globally.

In this study, we aimed to evaluate the association between elevated miR-210 and the survival of patients with cancer. We also planned to summarize the evidence for the use of miR-210 as a prognostic marker to predict other clinical outcomes.

233 identified studies

→ 218 out of scope

15 candidate studies

→ 3non-dichotomous; 2 HR missing

10 studies available for meta-analysis

Figure 1. Flow diagram of the study selection process.

Materials and methods

We attempted to report this meta-analysis in accordance with the Meta-Analysis of Observational Studies in Epidemiology guidelines [23].

Search strategy

We conducted a systematic literature search of the PubMed and EMBASE database from 1996 to 30 January 2014 by using the following search terms: 'miR-210 and cancer', 'miR-210 and cancer prognosis' and 'microRNAs and cancer prognosis'. Studies were included in this meta-analysis if they met the following criteria: (1) any type of carcinoma; (2) expression of miR-210 in tissue or serum; and (3) association between miR-210 expression levels and survival outcome. Non-English articles, review articles or letters and laboratory studies were excluded. Studies detected the effects of miR-210 on cancer outcome, but without dichotomous miR-210 expression levels and key information such as hazard ratio (HR), 95% CI and P value, were still kept in systematic review but not in the meta-analysis.

Two authors carefully checked the information such as titles, abstracts, full texts and reference lists of all of the eligible articles from all of the publications. Any disagreements were resolved by consensus. Furthermore, references from the relevant literature, including all of the identified studies, reviews and editorials, were manually searched. Additional information was requested from authors if needed.

Quality assess

All the studies were systematically assessed according to a critical review checklist of the Dutch Cochrane Centre proposed by MOOSE to ensure their quality [23]. The key points of the current checklist included are as follows: study population and origin of country, carcinoma type, study design, outcome assessment, measurement of miR-210, cut-off of miR-210 and sufficient period of follow-up. Studies which did not satisfy all these points were excluded to avoid compromised quality of the meta-analysis.

Data extraction and conversion

The extracted data mainly included first author's last name, publication year, studied population, miR-210 assessment and cut-off definition and HR of elevated miR-210 for overall survival (OS), distant-free survival (DFS), recurrence-free survival (RFS) or progressive-free survival (PFS), as well as their 95% confidence interval (CI) and P value. The simplest way to collect HR and their 95% CI is from the original article or from authors, with an HR > 1 indicating a poorer outcome. But most studies did not provide these statistical variables directly. If only Kaplan-Meier curves are available, data were extracted from the graphical survival plots and estimation of the HR was then performed as the previously described methods [24, 25].

Statistical analysis

HR was used as the common measure of association across studies, and RR was directly considered as HR. When there are more than four studies reported the same disease or survival analysis, we performed a subgroup analysis. Homogeneity of effect size across studies was tested by Q statistics at the P < 0.05 level of significance. We also calculated the I-squared statistic. A random effect model (Der Simonian and Laird method) was applied if heterogeneity existed (P < 0.05), while the fixed effect model was utilized in the absence of between-study heterogeneity (P ≥ 0.05). Potential publication bias was assessed by Begg's funnel plots and Egger's regression test [26]. All above calculations were performed using 'STATA: Data Analysis and Statistical Software' V12.0 (http://www.stata.com/).

Results

The primary search obtained 233 records for miR-210 and cancer in PubMed and EMBASE.

Table 1. Summary table of the 10 included studies and the 5 studies lack of HR data

Study	Origin of population	Study design	Disease	N	Stage	MiR-210 assay	Survival analysis	Hazard ratios	Follow-up, months
Camps 2008	UK	R	BC	219	I-III	qRT-PCR	OS, DFS	Reported	120
Rothé 2011	UK	R	BC	73	I-III	qRT-PCR	OS, RFS	Reported	120
Volinia 2012	USA	R	BC	58	-	qRT-PCR	OS	Reported	180
Radojicic 2011	Greece	R	BC	49	-	qRT-PCR	OS, DFS	K-M	120
Cai 2013	China	R	OSA	92	-	qRT-PCR	OS, PFS	Reported	82 (10-133)
Neal 2010	Australia	R	cc RCC	31	I-IV	qRT-PCR	OS	K-M	150
McCormick 2012	UK	R	cc RCC	41	I-III	qRT-PCR	OS	Reported	120
Greither 2009	Germany	R	PDAC	43	-	qRT-PCR	OS	Reported	15.99 (1-61)
Gee 2010	UK	R	HNSCC	46	I-IV	qRT-PCR	OS, RFS	K-M	41 (1-53)
Lai 2013	China	R	GBM	125	I-IV	qRT-PCR	OS, PFS	Reported	120
Greither 2011	Germany	R	STS	78	I-IV	qRT-PCR	OS	AP	120
Qiu 2013	-	R	GBM	458	-	qRT-PCR	OS, PFS	Reported	130
Osugi 2012	Japan	R	NSCLC	110	-	qRT-PCR	DFS	-	-
Duncavage 2009	USA	R	LS-NSCLC	44	I-II	qRT-PCR	OS	-	60
Ellermeier 2013	USA	R	CRC	31	-	qRT-PCR	OS	-	-

Study design is described as retrospective (R). BC, breast cancer; OSA, osteosarcoma; cc RCC, clear cell renal cell cancer; PDAC, pancreatic ductal adenocarcinomas; HNSCC, head and neck squamous cell carcinoma; GBM, glioblastoma multiforme; STS, soft-tissue sarcoma; NSCLC, non-small cell lung cancer; LS-NSCLC, Low stage (I and II) non-small cell lung cancer; CRC, colorectal cancer; qRT-PCR, quantitative real-time PCR; OS, overall survival; DFS, disease-free survival; RFS, relapse-free survival; PFS, progress-free survival; K-M, Kaplan-Meier survival curves; AP, author provided; -, not reported.

218 studies were excluded because they were review articles, letters, non-English articles, laboratory studies or studies irrelevant to our analysis. Of the 15 candidate studies, three studies did not deal with miR-210 expression data as a dichotomic variable [12, 18, 22]; and two others lacked of the key HR data [13, 15]. Eventually, our meta-analysis was carried out for the remaining 10 studies. A flow chart showing the study selection process is presented in **Figure 1**.

The main features of eligible studies are summarized in **Table 1**. We collected data from 10 studies including a total of 777 participants from the United States, the United Kingdom, Australia, Greece, Germany, China, and Japan. All of these studies were retrospective in design. The patients were of 6 kinds of carcinomas, including breast cancer, osteosarcoma, clear cell renal cancer, glioma, pancreatic ductal adenocarcinomas, head and neck squamous cell carcinoma. QRT-PCR was widely used in all the relevant studies to assess the miR-210 expression. Cancerous tissues were usually examined to determine miR-210 expression level, while serum samples were tested in one study [21]. Notably, the cut-off values of

miR-210 were different in the studies, with median was applied in nine studies and mean in only one study.

For studies evaluating OS, there appeared to have heterogeneity between studies for miR-210 ($P < 0.05$). Hence, a random model was used to calculate a pooled HR and its 95% CI. We found that higher expression levels of miR-210 significantly predicted poorer OS, with the pooled HR being 2.41 (95% CI: 1.31-4.44) (**Figure 2A**). For each study, the *P* value varied from 0.189 to < 0.001, and two studies had a *P* value above 0.05, which was not statistically significant. For studies evaluating RFS, DFS or PFS, a fixed model was applied because there was no heterogeneity between studies. MiR-210 expression was also significantly correlated with RFS/DFS/PFS, with the combined HR being 2.84 (95% CI: 2.10-3.84) (**Figure 2B**). There were 4 of included studies recruited breast cancer patients, and then subgroup analysis was performed in breast cancer. Heterogeneity also existed in breast cancer studies. Respectively, the HR of OS and DFS/RFS/PFS were 4.34 (95% CI: 1.63-11.55) (**Figure 3A**) and 3.31 (95% CI: 1.96-5.59) (**Figure 3B**), which were statistically significant.
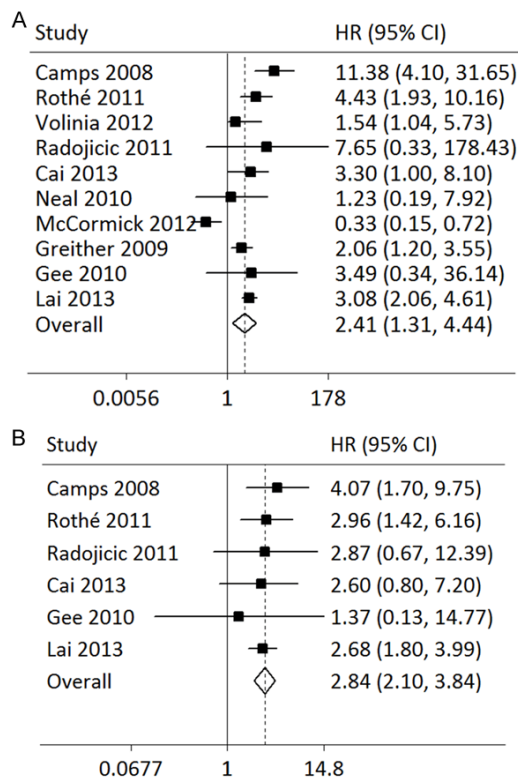
Figure 2. Forrest plots of studies evaluating hazard ratios of high miR-210 expression as compared to low expression. Survival data are reported as over-all survival (OS) (A) and relapse-free survival (RFS), disease-free survival (DFS) or progress-free survival (PFS) (B).

There were other 4 of included studies which patients were from the United Kingdom, and subgroup analysis showed that the HR of OS was 2.673 (95% CI: 0.44-16.18) (Figure 4). It means that high express of miR-210 is associated with worse patient OS whom were from the United Kingdom, however, this report did not reach statistical significance.

Finally, publication bias of the included studies was evaluated by funnel plots and Egger's tests. As shown in Figure 5A and 5B, the funnel plots showed no obvious asymmetry. In OS and DFS/RFS/PFS of all cancers meta-analysis, the P values of Egger's regression intercepts were 0.929 and 0.891, respectively. Therefore, no evidence for significant publication bias existed in the meta-analysis, because their P values were greater than 0.05. Since there are less than five prognostic studies in breast cancer, publication bias of the included studies was not conducted.
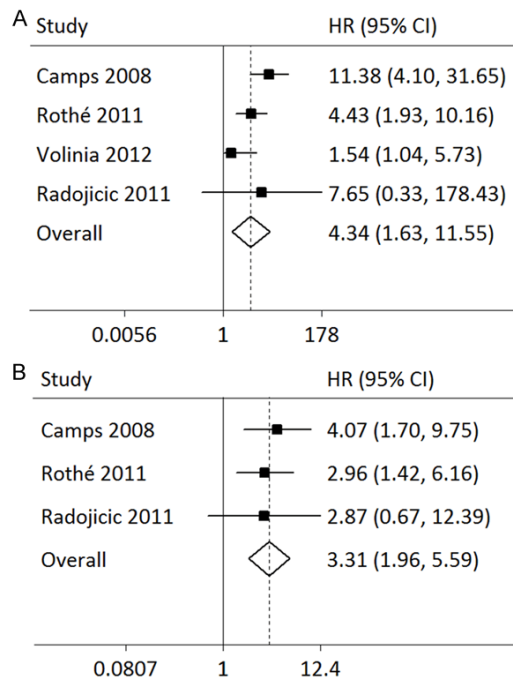

Figure 3. Forrest plots of OS (A) and RFS or DFS (B) in breast cancer.

Figure 4. Forrest plots of OS which patients were from the United Kingdom.

Discussion

It is unquestionable that miRNAs are important cancer biomarkers, thus there is growing interest in the field of miRNA research. Previous researches had shown that elevated expression of both miR-21 and miR-155 indicate a worse outcome within a wide variety of cancers [27, 28]. The prognostic values of miR-210 have also been proven in a recent meta-analysis on breast cancer [29]. Nevertheless, its data collection standards were not identical with our analysis. So we carry out a subgroup analysis in
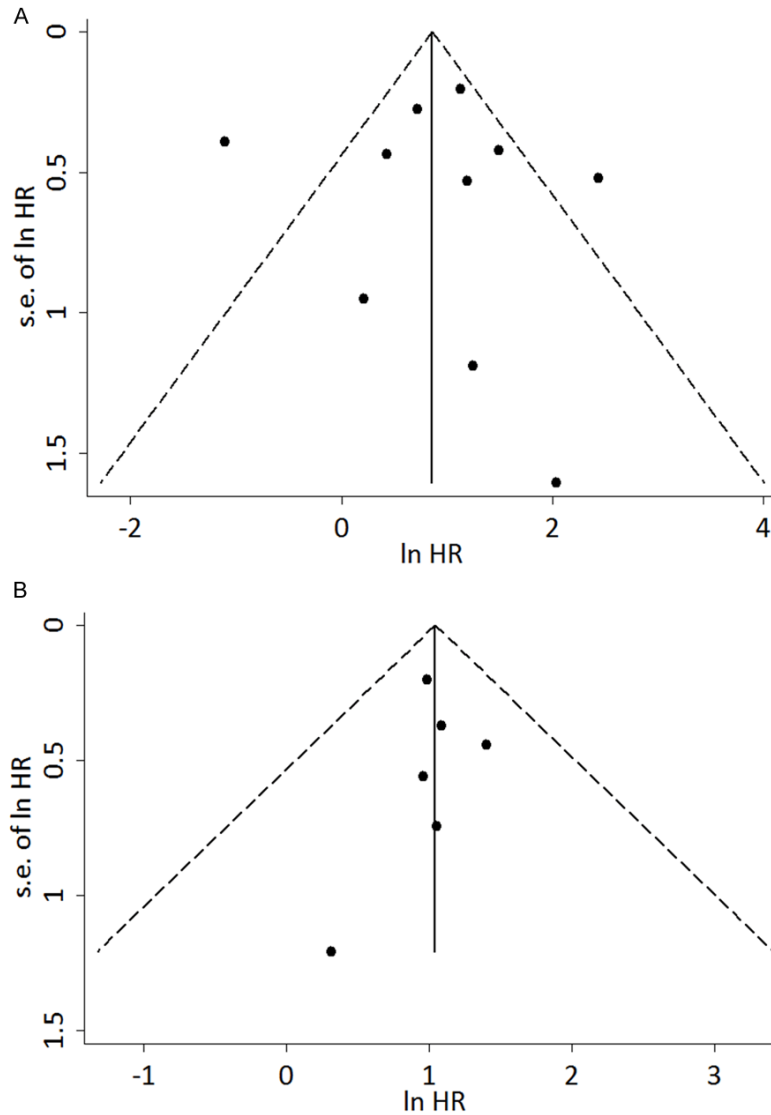
Figure 5. Funnel plots of studies included in the meta-analyses of all cancers. A. OS; B. RFS/DFS/PFS.

in clear cell renal cancer was associated with better prognosis, reduced proliferation and lower grade/stage of tumors [17].

In this study, we firstly performed a comprehensive and detailed meta-analysis to summarize the predictive role of miR-210 for survival in patients with a variety of carcinomas. The pooled results of HRs for OS and DFS/RFS/PFS were 2.41 and 2.84, respectively. We also conducted a subgroup analysis suggesting that higher expression of miR-210 correlated with worse OS in breast cancer (HR = 4.34). From the empirical analysis, HR > 2 is considered strongly predictive [32]. Consequently, this meta-analysis did show that the elevated expression of miR-210 indicated poor survival in patients with a variety of carcinomas, especially in breast cancer. It also did show that the elevated expression of miR-210 and OS which patients were from the United Kingdom (HR = 2.67) was not statistically significant, because of our limited data.

breast cancer based on the above criteria by reselecting relevant literature.

Hypoxia is involved in diverse pathological conditions including tissue ischemia, inflammation, and solid tumors [30]. As a widely accepted biomarker, miR-210 is the miRNA which is mostly related with tumor hypoxia, and a recent study reported that miR-210 is the most upregulated microRNA in lung cancer [31]. Besides, miR-210 is correlated with many other carcinomas, most of which have a worse outcome with elevated expression of miR-210. However, others found that increased miR-210

Though our data demonstrated that miR-210 was a promising biomarker of cancers, some potential limitations of this study should be taken into account. First, the number of cancer types in our meta-analysis is only six, which may not be applicable for other carcinomas. Therefore, we strongly recommend that more prognostic studies on miR-210 be conducted in more types of tumors. Second, although the pooled risks of miR-210 for OS and DFS/RFS/PFS were statistically significant with considerable high HRs (2.41 and 2.84), there was only one study in each type of cancers except breast cancer. Owing to our limited data, it is difficult

to do a subgroup analysis for other type of cancers. Third, the definition in miR-210 cut-off is ambiguous. Although most of them defined median as the cut-off of elevated miR-210 expression, the accurate values may be various in the different study populations. So this meta-analysis could not establish the exact cut off. Fourth, marked heterogeneity appeared in OS of all cancers because of the methodological restrictions. The heterogeneity was probably due to the different characteristics of patients (e.g. age, tumor stage, race or population), the cancer type, the cut-off value of miR-210, the duration of follow-up and others. When there are confounding factors in our data, we try to weaken their effects by using a random effect model. Finally, though our data shows that miR-210 is very promising for prognostic prediction, its clinical mechanism and application warrant exploration. Some colleagues suggested a way to combine two or three miRNAs to predict survival, such as miR-155 in coalition with miR-21 [33], or other miRNAs. So it is probably more reliable to combine miR-210 with other miRNAs to improve its prognostic power.

In conclusion, the elevated expression of miR-210 was significantly related with low survival rate in patients with different cancers. But the role of miR-210 in routine clinical management of cancer remains unevaluated. More clinical investigations should be carried out before the practical application of miR-210 in prognosis of cancer, especially a specific type of cancer.

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Disclosure of conflict of interest

None.

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