Original Paper

Evaluating the Prognostic Value of microRNA-203 in Solid Tumors Based on a Meta-Analysis and the Cancer Genome Atlas (TCGA) Datasets

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Key Words

MicroRNA-203 • Tumor • Prognosis • TCGA • Meta-analysis

Abstract

Background: It has been reported that miR-203 expression was aberrant in various types of cancers, and it could be used as a prognostic biomarker. Therefore, in this study, we aimed to evaluate the prognostic value of miR-203 expression in solid tumors by using meta-analysis and The Cancer Genome Atlas (TCGA) datasets. **Methods:** By doing a literature research in PubMed, Embase and the Cochrane Library (last update by December 2016), we were able to identify the studies assessing the prognostic role of miR-203 in various tumors. We then used TCGA datasets to validate the results of meta-analysis. **Results:** 33 studies from 26 articles were qualified and enrolled in this meta-analysis. Pooled analyses showed that higher expression of miR-203 in tissues couldn't predict poor overall survival (OS) and progression-free survival (PFS) in solid tumors. However, the results of subgroup analyses revealed that the upregulation of tissue miR-203 expression was associated with poor OS in colorectal cancer (hazard ratio (HR)=1.81, 95% confidence intervals (CI) 1.31-2.49; $P<0.001$), pancreatic cancer (HR=1.19, 95% CI 1.09-1.31; $P<0.001$) and ovarian cancer (HR=1.85, 95% CI 1.45-2.37; $P<0.001$); but it had opposite association in liver cancer (HR=0.52, 95% CI 0.28-0.97; $P=0.040$) and esophageal cancer (HR=0.41, 95% CI 0.25-0.66; $P<0.001$). Based on TCGA datasets, we found the same results for pancreatic cancer and esophageal cancer, but not for colorectal cancer and liver cancer. Moreover, patients with high circulating miR-203 in blood had significantly poor OS and PFS in colorectal cancer and breast cancer. **Conclusion:** Our study showed that the prognostic values of tissue miR-203 varied in different tumor types. In addition, the upregulation of circulating miR-203 in blood was associated with poor prognosis in colorectal cancer and breast cancer.

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Introduction

MicroRNAs (miRNAs) are a class of endogenously expressed small non-coding RNAs with a length of 19-25 nucleotides. They negatively regulate gene expression via base-pairing with target mRNAs (usually in the 3' untranslated region) [1]. MiRNAs have been shown to play important roles in cellular growth, differentiation, proliferation, metastasis, migration, and apoptosis. Recently, many studies have reported that numerous miRNAs were abnormally expressed in tumors and were often associated with diagnosis, clinicopathological features, prognosis and response to clinical therapies [2].

MiR-203, located at the chromosome 14q32.33, is known as a skin-specific miRNA and involved in regulating the embryonic epidermal differentiation and building the protective layer of skin [3]. Recently, it has been shown that miR-203 is a putative tumor suppressor and regulates tumor cell proliferation, invasion and tumor angiogenesis by targeting several genes, such as Bmi-1, CKAP2, LASP1, BIRC5, WASF1, ASAP1, SNAI1/2, RUNX2, ZEB1/2 and AKT2 [4-12]. Some studies have demonstrated that low miR-203 expression was significantly associated with poor prognosis in some tumors, such as liver cancer [13, 14], esophageal cancer [15, 16], head and neck squamous cell carcinomas (HNSCC) [17, 18], melanoma [19], bladder cancer [20], cervical cancer [21], non-small cell lung cancer (NSCLC) [22], glioma [23] and gastric cancer [24]. However, some other studies reported negative or even opposite results [25-38]. Therefore, the prognostic value of miR-203 for cancer patients is still unclear. In this study, we conducted a comprehensive and objective meta-analysis to evaluate the prognostic value of miR-203 expression in tissues and in bloods. Moreover, we used an independent miR-203 microarray dataset from The Cancer Genome Atlas (TCGA) to validate the results of meta-analysis.

Materials and Methods

The meta-analysis was carried out in accordance with the Observational Studies in Epidemiology (MOOSE) guidelines [39].

Literature searching strategy

We carefully searched for literatures using the online databases including PubMed, Embase and the Cochrane Library (last update by December 12, 2016). The keywords used in the search were "microRNA-203 OR miR-203 OR miR-203a" (all fields) AND "tumor OR tumour OR cancer OR neoplasm OR carcinoma" (all fields). We did not set any advanced limitations. The reference lists of full-text articles were also screened to avoid omitted studies. Two authors conducted the search independently (Y. Shao and W. Gu).

Inclusion and exclusion criteria

Literatures were considered eligible if they met the following criteria: (1) measuring the expression of miR-203 in cancer tissues or blood; (2) investigating the relationship between miR-203 expression level and survival outcome; (3) providing sufficient data to estimate the hazard ratio (HR) and 95% confidence intervals (CI). Articles were excluded based on the following standards: (1) including case reports, reviews, conference abstracts, letters and animal trials; (2) when more than one study reported on the same patient cohort, only the most recent or complete study was included. Two reviewers evaluated the titles and abstracts of the uncertain articles independently and excluded the irrelevant ones. The full text of enrolled articles was carefully examined for comprehensive evaluation. Disagreements were resolved by discussion with a third reviewer (X. Song).

Data extraction and quality assessment

Two authors extracted the data independently. The relevant information included the first author's surname, publication year, country, tumor type, sample number, tumor stage, lymph node metastasis, follow-up time, detected sample, miR-203 assessment methods and cut-off value, HR of miR-203 expression for overall survival (OS) and progression-free survival (PFS), and the corresponding 95% CIs. If a study

reported the results of both univariate and multivariate analyses, multivariate analysis was selected because it considered the confounding factors and was more accurate.

The quality of each study was assessed independently by two researchers according to the Newcastle-Ottawa Quality Assessment Scale (NOS) [40].

The extraction and analysis of TCGA datasets

The miR-203 expression and clinical data for TCGA solid tumors (including adrenocortical cancer, bile duct cancer, bladder cancer, breast cancer, cervical cancer, colorectal cancer, endometrioid cancer, esophageal cancer, HNSCC, kidney cancer, liver cancer, lung cancer, glioma, melanoma, ovarian cancer, pancreatic cancer, prostate cancer, sarcoma, gastric cancer, testicular cancer, thyroid cancer and mesothelioma) were extracted from the UCSC Xena website (<https://xenabrowser.net/heatmap/>) [41]. MiRNA expression was measured by IlluminaHiSeq platform. There were 8568 subjects that have both survival and miR-203 expression date in TCGA Pan-cancer datasets [42]. The miR-203 expression levels were dichotomized into high expression and low expression groups based on the receiver operating characteristic (ROC) curves with Youden's index correction [43]. The survival curves were plotted and tested using the Kaplan-Meier method and log-rank test. The influence of miR-203 expression on survival was assessed by the Cox proportional hazards model. Statistical analysis was carried out by SPSS 17.0 software (SPSS, Chicago, IL).

Statistical analysis

The statistical analysis followed our previous study [44]. The high and low expression of miR-203 was defined according to the cut-off values provided by the articles. We used HRs and the corresponding 95% CIs to calculate the pooled data. If the statistical variables were described in the study, we then directly used them in our analysis. Otherwise, the data were extracted from graphical survival plots according to the methods described by Tierney [45]. The data from Kaplan-Meier survival curves were read by Engauge Digitizer version 4.1, and three researchers read the curves independently to reduce reading variability. We also sent e-mails to the corresponding authors of the eligible studies to request original data if needed. An observed HR greater than 1 indicated a poor prognosis in patients with miR-203 overexpression, and HR less than 1 suggested a better prognosis. We used the Chi-square test and I^2 statistic to evaluate the heterogeneity [46, 47]. A random-effects model (the DerSimonian-Laird method) was used if the P value was less than 0.05 and/or I^2 exceeded 50%. Otherwise, the fixed-effects model (the Mante-Haenszel method) was used. Subgroup analysis and meta-regression were used to explore the factors contributing to heterogeneity. Publication bias was analyzed by funnel plot, Begg's and Egger's tests. All the analyses were performed by STATA vision 12.0 software (Stata Corporation, College Station, TX, USA). P value less than 0.05 was considered statistically significant, otherwise it would be specified.

Results

Study characteristics

By using the described searching strategy, we primarily collected 813 references. Then we screened the title, abstract, publication type and full text of each publication, and found 28 articles investigating the relationship between miR-203 expression and patients' outcome in various tumors. But 2 of the 28 articles were excluded because they did not provide sufficient data to calculate the HRs and 95% CIs [48, 49]. Finally, 26 articles containing 33 studies, which were published between 2008 and 2016, were included in this meta-analysis (Fig. 1). The total number of patients included was 2997, ranging from 34 to 344 patients per study. Quantitative real-time polymerase chain reaction (qRT-PCR) was used to detect miRNA expression in all studies besides one. The miR-203 expression was mostly detected in tumor tissues, while in six studies it was tested in plasma or serum. The cut-off values of miRNA expression were different in each study, mainly including the optimal cut-off (the miRNA expression values were dichotomized into high expression and low expression groups based on receiver operating characteristic (ROC) curves with Youden's index correction [43]) and median level. HR was evaluated in eight studies and reported by text in other studies. In the primary reports, 14 studies showed a significant association between low miR-203 and poor

Fig. 1. Flow diagram of the study selection process.

Fig. 2. Forest plot of the relationship between high miR-203 expression in tissues and the overall survival (OS) of cancer patients, using a random-effects model.

prognosis, 15 studies provided the opposite result, and 4 studies found no prognostic value of miR-203 for tumor patients. The main information of the 26 eligible articles is shown in Table 1. The quality of each eligible article included in our meta-analysis was assessed according to NOS, and ranged from 4 to 8, with a mean of 6.4. The meta-analysis was based on both tissue and circulating miR-203 separately, because previous studies reported that there was no association between serum miR-203 and tissue miR-203 expression [26].

Meta-analysis of miR-203 expression in tissues

There were 24 articles including 27 studies that reported data about tissue miR-203 expression and the prognosis, which involved 2,408 cancer patients [13, 15-23, 26-31]. Since the result of meta-analysis exhibited obvious heterogeneity ($P < 0.001$, $I^2 = 88.3\%$) (Table 2), we used a random model to calculate the pooled HR and 95% CI from the 22 studies that

Table 1. The main characteristic of all studies in the meta-analysis. qRT-PCR - quantitative real-time PCR, ISH - in situ hybridization, OS - overall survival, PFS - progression-free survival, NR - not reported, NSCLC - non-small cell lung cancer, HNSCC - head and neck squamous cell carcinomas

Study	country	Tumor type	Sample number	Tumor stage I/II/III/IV	Lymph node -/+	Follow-up months	Detected sample	MR-203 assay	Cut-off value	Multivariate analysis	Survival analysis
Madhavan 2016 [25]	Germany	breast cancer	237	0/0/0/237	NR	NR	serum	qRT-PCR	lower	NO	OS, PFS
Wang 2015 [19]	China	melanoma	148	I-II/25/III-IV/36	73/75	over 60	tissue	qRT-PCR	quartile	NO	OS
Hur 2015 [26]	Japan	colorectal cancer	186	45/60/41/40	109/77	over 40	serum	qRT-PCR	optimal	YES	OS
Hur 2015 [26]	Japan	colorectal cancer	154	37/48/39/30	87/67	over 40	tissue	ISH	optimal	NO	OS
Hur 2015 [26]	Japan	colorectal cancer	144	31/45/37/31	NR	NR	serum	qRT-PCR	optimal	NO	OS
Zhang 2015 [20]	China	bladder cancer	108	NR	45/63	median 51.5	tissue	qRT-PCR	optimal	YES	OS, PFS
Liu 2016 [15]	China	esophageal cancer	119	I-II/63/III-IV/56	26/93	median 36	tissue	qRT-PCR	optimal	NO	OS
Huang 2016 [27]	China	prostate cancer	44	I-II/19/III-IV/25	NR	over 36	tissue	qRT-PCR	optimal	NO	OS
Safioleslam 2015 [21]	Iran	cervical cancer	54	NR	35/19	over 60	tissue	qRT-PCR	optimal	NO	OS
Li 2015 [13]	China	liver cancer	148	38/44/66/0	NR	mean 26	tissue	qRT-PCR	median	NO	OS
Jiang 2016 [22]	China	NSCLC	57	NR	NR	tissue	tissue	qRT-PCR	median	NO	OS
Jong 2015 [17]	Netherlands	HNSCC	34	NR	NR	mean 45.6	tissue	qRT-PCR	median	YES	PFS
Liu 2015 [29]	China	liver cancer	95	I-II/22/III-IV/73	NR	mean 33.1	tissue	qRT-PCR	median	NO	PFS
Hezova 2015 [16]	Czech Republic	esophageal cancer	22	NR	NR	NR	tissue	qRT-PCR	median	NO	OS
Hu 2014 [28]	China	renal cancer	40	NR	23/17	NR	tissue	qRT-PCR	optimal	NO	OS
Tao 2014 [30]	China	colorectal cancer	90	8/47/32/2	34/56	median	tissue	qRT-PCR	median	NO	OS
Tian 2014 [18]	China	HNSCC	56	I-II/24/III-IV/32	28/28	over 60	tissue	qRT-PCR	median	NO	OS
Wang 2013 [31]	China	ovarian cancer	156	26/22/70/38	70/86	median	tissue	qRT-PCR	optimal	NO	OS, PFS
He 2013 [23]	China	glioma	112	NR	NR	median	tissue	qRT-PCR	median	NO	OS
Bovell 2013 [32]	USA	colorectal cancer	344	I-II/184/III-IV/20	189/146	median	tissue	qRT-PCR	over normal	YES	OS
Madhavan 2012 [33]	Germany	breast cancer	176	IV/20	NR	180	serum	qRT-PCR	lower	NO	OS, PFS
Chen 2012 [14]	China	liver cancer	66	I-II/59/III-IV/7	NR	median	tissue	qRT-PCR	quartile	YES	OS, PFS
Ikenaga 2010 [34]	Japan	pancreatic cancer	107	NR	37/76	49	tissue	qRT-PCR	median	YES	OS
Greither 2010 [35]	Germany	pancreatic cancer	50	NR	NR	14.7	tissue	qRT-PCR	median	YES	OS
Schetter 2008 [36]	USA	colorectal cancer	84	8/29/36/10	NR	15.3	tissue	qRT-PCR	higher	YES	OS
Schulz 2012 [37]	Denmark	pancreatic cancer	138	NR	NR	68	tissue	qRT-PCR	quartile	NO	OS
Azizomohammadi 2016 [38]	Iran	ovarian cancer	55	I-II/21/III-IV/34	23/32	NR	tissue	qRT-PCR	median	NO	OS
Liu 2016 [24]	China	gastric cancer	127	I-II/56/III-IV/71	20/107	median 61.2	tissue	qRT-PCR	optimal	NO	OS

provided OS of patients. The results showed that low miR-203 expression was not associated with worse OS in solid tumors, with the pooled HR of 0.85 (95% CI 0.64-1.14, $P=0.372$) (Table 2; Fig. 2). As the five studies that reported PFS displayed obvious heterogeneity ($P<0.001$, $I^2=93.7\%$) (Table 2), we also calculated the pooled HR using a random model. The pooled HR indicated no significant relationship between tissue miR-203 expression and PFS (HR=0.48, 95% CI 0.17-1.33; $P=0.159$) (Table 2).

Considering the heterogeneity among the studies, three subgroup analyses were performed, in which the patients were stratified by tumor type, analysis type and cut-off value. All the pooled HRs and corresponding 95% CIs of the subgroups are shown in Table 2. In the subgroups based on tumor type, we found that upregulation of miR-203 was associated with worse OS in colorectal cancer (HR=1.81, 95% CI 1.31-2.49; $P<0.001$), pancreatic cancer (HR=1.19, 95% CI 1.09-1.31; $P<0.001$) and ovarian cancer (HR=1.85, 95% CI 1.45-2.37;

Table 2. The pooled associations between different groups of miR-203 overexpression and the prognosis of patients. qRT-PCR - quantitative real-time PCR, ISH - in situ hybridization, OS - overall survival, DFS - disease-free survival, PFS - progression-free survival, HR - hazard ratio, CI - confidence interval, fixed - fixed-effects model, random - random-effects mode

Outcome subgroup (sample type)	No. of patients	No. of studies	HR (95%CI) - model	P value	heterogeneity I ² (%)	P	Publication bias Begg's P	Egger's P
OS (tissue)	2279	22	0.85 (0.64-1.14) - random	0.372	88.6	<0.001	0.955	0.164
Tumor type								
Colorectal cancer	672	4	1.81 (1.31-2.49) - fixed	<0.001*	10.6	0.340	0.308	0.195
Pancreatic cancer	295	3	1.19 (1.09-1.31) - fixed	<0.001*	38.5	0.197	0.296	0.455
Liver cancer	214	2	0.59 (0.43-0.82) - fixed	0.002*	48.6	0.155	-	-
Esophageal cancer	141	2	0.41 (0.25-0.66) - fixed	<0.001*	0	0.416	-	-
Ovarian cancer	211	2	1.85 (1.45-2.37) - fixed	<0.001*	0	0.344	-	-
Others	746	9	0.51 (0.30-0.88) - random	0.014*	84.8	<0.001	0.466	0.274
Analysis type								
Univariate	1595	16	0.77 (0.55-1.07) - random	0.122	89.6	<0.001	0.558	0.105
Multivariate	684	6	1.13 (0.54-2.36) - random	0.747	87.1	<0.001	0.452	0.732
Cut-off value								
Median	967	12	0.69 (0.46-1.04) - random	0.074	88.1	<0.001	0.373	0.080
Optimal cut-off	765	7	0.99 (0.52-1.89) - random	0.978	91.1	<0.001	0.707	0.762
Others	547	3	1.37 (0.41-4.62) - random	0.613	91.4	<0.001	0.296	0.315
PFS (tissue)	459	5	0.48 (0.17-1.33) - random	0.159	93.7	<0.001	0.806	0.220
OS (blood)	743	4	2.23 (1.67-2.97) - fixed	<0.001*	0	0.996	0.734	0.746
Tumor type								
Colorectal cancer	330	2	2.20 (1.50-3.24) - fixed	<0.001*	0	0.835	-	-
Breast cancer	413	2	2.26 (1.47-3.48) - fixed	<0.001*	0	0.996	-	-
PFS (blood)	401	2	1.29 (1.03-1.62) - fixed	0.025*	0	0.459	-	-

Fig. 3. Forest plot of the relationship between high miR-203 expression in tissues and the overall survival (OS) of patients with colorectal cancer, pancreatic cancer, liver cancer and esophageal cancer, using a fixed-effects model.

$P < 0.001$) (Table 2; Fig. 3). However, for liver cancer (HR=0.52, 95% CI 0.28-0.97; $P=0.040$) and esophageal cancer (HR=0.41, 95% CI 0.25-0.66; $P < 0.001$), the association was opposite (Table 2; Fig. 3). There was only one study for each that evaluated the association between miR-203 expression and OS in melanoma, bladder cancer, prostate cancer, cervical cancer, NSCLC, renal cancer, gastric cancer, HNSCC and glioma. Therefore, these tumors were defined as "other cancers". The combined data from these nine studies showed that high miR-203 expression was associated with better OS prognosis (HR=0.51, 95% CI 0.30-0.88; $P=0.014$). We did not find any association between miR-203 and OS in subgroup analyses of cut-off value and analysis type.

Meta-analysis of circulating miR-203 expression in blood

A total of six studies reported the relationship between blood miR-203 expression and the prognosis of tumor patients [25, 26, 33]. As four of the six studies reported OS and

Fig. 4. Forest plot of the relationship between high circulating miR-203 expression in blood and the overall survival (OS) of patients with breast cancer and colorectal cancer, using a fixed-effects model.

Fig. 5. Funnel plots of the studies included in the two meta-analyses: (A) the overall survival based on tissue miR-203 expression (B) the overall survival based on blood miR-203 expression.

displayed no heterogeneity among them ($P=0.996$, $I^2=0\%$) (Table 2), we used a fixed model and found that patients with high miR-203 expression in blood had significantly poorer OS (HR=2.23, 95% CI 1.67-2.97; $P<0.001$) (Table 2; Fig. 4). The outcome of PFS reported by 2 studies were also pooled and analyzed, and the pooled HR revealed a positive association between high levels of miR-203 in blood and poor PFS (HR=1.29, 95% CI 1.03-1.62, $P=0.025$) (Table 2). In the subgroups based on tumor type, we found high miR-203 was associated with poor OS in colorectal cancer (HR=2.20, 95% CI 1.50-3.24; $P<0.001$) and breast cancer (HR=2.26, 95% CI 1.47-3.48; $P<0.001$) (Table 2).

Heterogeneity Analysis

The heterogeneity in OS analysis group, which included 22 studies, was very obvious ($P<0.001$, $I^2=88.3\%$) based on tissue miR-203 expression. Therefore, we conducted a meta-regression analysis to evaluate the possible factors related to the heterogeneity. The results showed that the heterogeneity was caused by tumor type ($P=0.032$) instead of cut-off value ($P=0.710$), analysis type ($P=0.273$), miR-203 detected method ($P=0.787$), country ($P=0.623$), follow-up time ($P=0.356$) or publication year ($P=0.788$).

Table 3. HRs and corresponding 95% CIs of miR-203 overexpression in solid tumors based on The Cancer Genome Atlas (TCGA) datasets. HNSCC - head and neck squamous cell carcinomas, HR - hazard ratio, CI - confidence interval

Tumor type	Sample size	MiR-203 assay	HR (95% CI)	P value
Solid tumor	8568	Illumina Hiseq	1.07 (0.98-1.16)	0.128
Adrenocortical cancer	80	Illumina Hiseq	0.39 (0.19-0.83)	0.014*
Bile duct cancer	34	Illumina Hiseq	1.13 (0.42-3.02)	0.814
Bladder cancer	330	Illumina Hiseq	1.58 (1.06-2.35)	0.025*
Breast cancer	1027	Illumina Hiseq	1.25 (0.87-1.81)	0.230
Cervical cancer	281	Illumina Hiseq	0.62 (0.34-1.14)	0.126
Colorectal cancer	558	Illumina Hiseq	1.12 (0.75-1.67)	0.574
Endometrioid cancer	519	Illumina Hiseq	1.48 (0.84-2.61)	0.171
Esophageal cancer	169	Illumina Hiseq	0.43 (0.19-0.99)	0.049*
HNSCC	504	Illumina Hiseq	0.73 (0.48-1.11)	0.137
Kidney cancer	807	Illumina Hiseq	1.42 (1.06-1.92)	0.020*
Liver cancer	330	Illumina Hiseq	1.42 (0.79-2.54)	0.237
Lung cancer	916	Illumina Hiseq	1.05 (0.83-1.32)	0.700
Glioma	451	Illumina Hiseq	0.57 (0.34-0.93)	0.026*
Melanoma	135	Illumina Hiseq	0.56 (0.27-1.16)	0.117
Ovarian cancer	468	Illumina Hiseq	1.04 (0.80-1.34)	0.771
Pancreatic cancer	161	Illumina Hiseq	2.18 (1.35-3.51)	0.001*
Prostate cancer	416	Illumina Hiseq	5.59 (0.66-47.49)	0.115
Sarcoma	300	Illumina Hiseq	1.84 (1.26-2.69)	0.002*
Gastric cancer	391	Illumina Hiseq	0.75 (0.53-1.05)	0.095
Testicular cancer	119	Illumina Hiseq	5.87 (0.53-64.81)	0.148
Thyroid cancer	500	Illumina Hiseq	2.59 (0.72-9.31)	0.144
Mesothelioma	72	Illumina Hiseq	0.49 (0.25-0.93)	0.029*

Fig. 6. Kaplan-Meier survival curves for cancer patients, stratified by miR-203 expression levels. The cancer types are (A) adrenocortical cancer, (B) bladder cancer, (C) esophageal cancer, (D) kidney cancer, (E) glioma, (F) pancreatic cancer, (G) sarcoma and (H) mesothelioma.

Publication Bias

We used funnel plots, Egger's and Begg's tests to evaluate the publication bias of the included studies. The funnel plots of the OS analysis that based on both tissue and blood miR-203 expression were almost symmetric, as shown in Figure 5. Consistently, in the 16 analysis groups, no significant publication bias was observed.

Validation by independent TCGA solid tumor datasets

To validate the results of the meta-analysis, we used tissue miR-203 expression data and the corresponding survival data from TCGA datasets to compare, which included 8568

patients with various types of cancer: adrenocortical cancer, bile duct cancer, bladder cancer, breast cancer, cervical cancer, colorectal cancer, endometrioid cancer, esophageal cancer, HNSCC, kidney cancer, liver cancer, lung cancer, glioma, melanoma, ovarian cancer, pancreatic cancer, prostate cancer, sarcoma, gastric cancer, testicular cancer, thyroid cancer and mesothelioma. The results showed that high miR-203 expression in tissues was not associated with worse OS in the pooled analysis on all the solid tumors (HR=1.07, 95% CI 0.98-1.16; $P=0.128$) (Table 3). However, in the analysis of single tumor types, we found that the upregulation of miR-203 was significantly associated with worse OS in pancreatic cancer (HR=2.18, 95% CI 1.31-2.49; $P=0.001$), bladder cancer (HR=1.58, 95% CI 1.06-2.35; $P=0.025$), kidney cancer (HR=1.42, 95% CI 1.06-1.92; $P=0.020$) and sarcoma (HR=1.84, 95% CI 1.26-2.69; $P=0.002$), while the association was opposite in esophageal cancer (HR=0.43, 95% CI 0.19-0.99; $P=0.049$), adrenocortical cancer (HR=0.39, 95% CI 0.19-0.83; $P=0.014$), glioma (HR=0.57, 95% CI 0.34-0.93; $P=0.026$) and mesothelioma (HR=0.49, 95% CI 0.25-0.93; $P=0.029$) (Table 3; Fig. 6). In the other tumor types, miR-203 expression was not associated with patients' prognosis (Table 3). Therefore, most of the results from TCGA are consistent with our meta-analysis, except for the ones in colorectal cancer and liver cancer.

Discussion

MiR-203 is located in a fragile chromosomal region that is frequently lost in T cell malignancies. Epigenetic silencing of miR-203 can enhance the activation of the BCR-ABL1 fusion gene, resulting in increased tumor cell proliferation. Also, epigenetic inactivation of miR-203 is frequently observed in a variety of tumors.

In breast cancer cells and prostate cells, MiR-203 can regulate EMT process by directly targeting the key EMT regulators, such as ZEB1/2 and SNAI1/2 [4, 6-10, 50]. Moreover, ectopic expression of miR-203 also delayed EMT induction and invasion by suppressing NUA1 expression [51]. Thereby, low miR-203 expression can promote EMT and disease progression. In addition, miR-203 also plays a crucial role in regulating stem cell self-renewal and proliferation. MiR-203 can strongly decrease the fraction of side population cells and colony formation by targeting Bmi-1 in esophageal cancer and leukemia stem cell [5, 52]. In osteosarcoma, inhibition of miR-203 stimulated cell growth by targeting TBK1 and RAB22A [53, 54]. Therefore, miR-203 is involved in tumor cell differentiation, proliferation and metastasis, and is thought to be a tumor suppressor. However, upregulation of miR-203 has been found in multiple tumor types including colorectal cancer [26, 32, 36], pancreatic cancer [34, 35, 37], renal cell carcinoma [28], breast cancer [25, 33] and ovarian cancer [31], indicating that miR-203 may also exhibit oncogenic potential, likely due to its ability to induce MET and promote metastatic colonization at distant sites [26]. Thus, it is controversial that whether miR-203 expression can be used as a prognostic biomarker in cancer.

In this meta-analysis, a total of 33 studies with 2,997 patients were enrolled. The results showed that the prognostic value of tissue miR-203 expression varied in different tumor types. The upregulation of miR-203 in tissues was significantly associated with worse OS in colorectal cancer (HR=1.81, 95% CI 1.31-2.49; $P<0.001$), pancreatic cancer (HR=1.19, 95% CI 1.09-1.31; $P<0.001$) and ovarian cancer (HR=1.85, 95% CI 1.45-2.37; $P<0.001$). Conversely, the downregulated miR-203 in tissues indicated poor OS in liver cancer (HR=0.52, 95% CI 0.28-0.97; $P=0.040$) and esophageal cancer (HR=0.41, 95% CI 0.25-0.66; $P<0.001$). In the analysis about blood miR-203 expression, the results showed that overexpression of miR-203 in blood was associated with poor prognosis (HR=2.23, 95% CI 1.67-2.97; $P<0.001$). In addition, we used the data from the TCGA datasets to validate the meta-analysis results. The same results were found in pancreatic cancer and esophageal cancer, but not in colorectal cancer and liver cancer. Previously, Frampton et al. found that miR-203 expression had prognostic significance in pancreatic cancer in a meta-analysis [55], which is consistent with our results and the TCGA pancreatic cancer datasets. Recently, Liang et al. also assessed the correlation between miR-203 expression and the prognosis of patients with solid tumors,

but their literature searching only included 13 studies with 1600 patients [56], which is not comprehensive enough to collect all the relevant evidence available from literature. Compared with their study, we included more studies and patients, thus providing stronger evidence.

Although our study provided robust evidence about the prognostic value of miR-203 expression, some limitations need to be acknowledged. First, two studies that explored the association between miR-203 expression and prognosis were not included in the meta-analysis because they did not provide available data. Secondly, the cut-off value used in each study was different so that a common cut-off value could not be set up. The inaccurate cut-off values may affect the evaluation of miR-203 as a prognostic biomarker. Thirdly, some of the HRs and CIs were calculated based on the data extracted from the survival curves, which inevitably brought small statistical errors. Fourthly, tumor type, cut-off value, analysis type, miR-203 detected method, country, follow-up time and publication year might also contribute to the heterogeneity. Finally, although no significant publication bias was detected in this meta-analysis, the results still need to be verified by a large number of studies.

In conclusion, based on the meta-analysis and TCGA datasets, our study demonstrated that the prognostic values of tissue miR-203 expression varied indifferent types of cancer. Low expression of miR-203 in primary tissues was significantly associated with poor survival in esophageal cancer, whereas high levels of miR-203 in tissues was associated with poor prognosis in pancreatic cancer. Furthermore, high circulating miR-203 expression indicated poor prognosis in colorectal cancer and breast cancer.

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Disclosure Statement

The authors disclose no potential conflicts of interest.

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