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ORIGINAL RESEARCH

The correlation between microRNA-221/222 cluster overexpression and malignancy: an updated meta-analysis including 2693 patients

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Background: Although miR-221/222 cluster plays an important role in many human malignancies, the correlation between miR-221/222 cluster overexpression and tumor prognosis remains controversial. Therefore, an updated meta-analysis was conducted to clarify its prognostic value in malignancy.

Methods: We conducted a search of literature in English electronic databases of PubMed, Embase, and Cochrane Library, and Chinese electronic databases of China Biology Medicine disc and China National Knowledge Infrastructure to obtain appropriate studies. Besides, we extracted hazard ratios (HRs) and 95% CIs to evaluate the strength of the correlations. In addition, the results of different subgroups analyses and publication bias test were also shown in this article.

Results: 32 publications, including 15 tumor types and 2,693 patients were embraced in this meta-analysis. The results of univariate (HR =1.69, 95% CI: 1.18–2.44, $P<0.01$) and multivariate (HR =2.10, 95% CI: 1.63–2.69, $P<0.01$) analyses revealed that miR-221/222 cluster high expression in various tumors was significantly associated with adverse overall survival (OS). Correspondingly, we also found subgroups analyses consisted of country, miR-221/222 cluster component, sample size, and test method have similar results.

Conclusion: miR-221/222 cluster overexpression was closely related to adverse OS in human carcinoma, while overexpression of miRNA-221/222 cluster could be viewed as a protection factor in prostate cancer. Blood-derived miR-221/222 cluster was not proper to assess OS.

Keywords: MiRNA-221/222 cluster, cancer, prognosis, meta-analysis

Introduction

miRNAs are an abundant class of endogenous small non-coding RNAs. Since the discovery of miRNAs, emerging studies have demonstrated that miRNAs could serve as oncogenes or tumor suppressors participating in initiation and progression of various cancers.[1] It is well documented that miRNAs can inhibit translation and/or induce degradation of target mRNAs, thereby negatively regulated gene expression. The misexpression of miRNAs has a great impact on extensive biological functions consisting of cell differentiation, apoptosis, tumorigenesis, and metabolism.[2] Thus, aberrantly expressed miRNAs can be viewed as a new type of biomarkers for monitoring therapeutic efficacy and predicting prognosis.

Two highly homologous miRNAs (miR-221, miR-222), commonly acting as a gene cluster (miR-221/222 cluster), have been extensively studied in human malignancies.[3] miR-221/222 cluster overexpression was observed in hepatocellular cancer, pancreatic cancer (PC), breast cancer (BC), etc[4–6]; while the lowly expressed miR-221/222

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cluster was found in tongue squamous cell carcinoma and prostate cancer (PCa).[7,8] Several studies have shown that the expression levels of miR-221/222 cluster have close connections with occurrence, progression, and prognosis in several tumors. For instance, a receiver operating characteristic curve analysis conducted by Eissa et al revealed that the higher miR-221 expression, the worse 5-year relapse-free survival (RFS) in patients with BC (P=0.0124)[6]; a recent study indicated that overall survival (OS) of higher miR-221 expression group was obviously lower than that of the lower miR-221 expression group (P<0.05).[9] Whereas, according to the log-rank test, including 125 triple-negative BC (TNBC) patients, Deng et al found that patients with high miR-221 expression have a better 5-year disease-free survival (DFS).[10] The aforementioned studies showed that miR-221/222 cluster could be potentially used in predicting the prognostic value of cancer. Meanwhile, the details of over-expressed miR-221/222 cluster's prognostic value in various cancer types are still controversial.

Meta-analysis is capable to obtain relatively accurate estimation by integrating all available evidence to explore authentic and comprehensive results.[11] The only meta-analysis regarding the prognostic value of miRNA-221/222 cluster in cancers was published in 2013 by Wang et al.[12] Thus, we carried out an updated meta-analysis with larger sample size, more cancer types, more ethnicities, and different analysis models to verify the previous conclusions and especially uncover some novel findings, which may contribute to the exploration of new promising biomarkers for assessing the therapeutic efficacy and prognostic value of different types of cancers.

Methods

Search strategy

To search eligible studies, we explored literature resources consisting of PubMed, Cochrane Library, Embase, Chinese National Knowledge Infrastructure, and Chinese Biomedical Literature database, with the terms ("microRNA OR miRNA OR miR-221 OR miR-222 OR miR-221/222 cluster OR miR-221/222 family"), ("survival OR prognosis OR prognostic") and ("cancer OR tumor OR tumor OR neoplasm OR neoplasma OR neoplasia OR carcinoma OR Malignancy"). The deadline for current investigation was on January 30, 2018. Meanwhile, the language of publication was only limited to English and Chinese. To avoid omissions as much as possible, we checked through the references list in search of potentially relevant researches.

Inclusion and exclusion

Available studies must obey the following criteria: 1) Clinical study about the correlation of miR-221/222 family with human cancer prognosis; 2) Study with relevant data of hazard ratios (HRs) with corresponding 95% CIs. Besides, studies met one of the criteria should be excluded as follows: 1) Studies with deficient data; 2) Duplicate studies (we select the study with relatively complete data); 3) Articles with other types, such as reviews and abstracts; and 4) Cell lines or animal models as research objects.

Data extraction

At the criteria of inclusion and exclusion, the corresponding data of qualified studies were extracted. If we noticed disagreements, a discussion would be conducted by PZ and MZ, or further reviewed by RH. The data, including first author, publication year, research country, test method, cancer type, miRNAs category, sample source, survival outcome, HR (95% CI), sample size, and the cutoff value were extracted to assess the tumor prognosis. Moreover, patient sources came from Asia, Africa, Europe, and North America. In situ hybridization (ISH) and reverse transcription-polymerase chain reaction (RT-PCR) were included in test methods; sample sources were divided into tissue, formalin-fixed and paraffin-embedded (FFPE), and blood; sample sizes included ≥100 and <100 groups; we just divided cancer types into 3 groups of hepatocellular carcinoma (HCC), non-small cell lung cancer (NSCLC), and others due to insufficient data; analyses methods included univariate and multivariate analyses. Patients' prognostic outcomes consisted of OS, DFS, RFS, and progression-free survival (PFS).

Data analysis

STATA (Version 12.0) and Review Manager (RevMan 5) softwares were utilized in this study. HR and corresponding 95% CI were extracted to evaluate the potential prognostic value of miR-221/222 cluster overexpression in human malignancy. Moreover, chi-square-based Q and I^2 tests have been applied in this study to assess the heterogeneity. (I^2<25% means no heterogeneity, I^2>50% means extreme heterogeneity).[13] Generally, we used fixed-effects model in studies with no or moderate heterogeneity. When I^2>50% or P<0.01 for Q test occurred in studies, we always used random-effects model to avoid obvious heterogeneity.[14] Publications were individually deleted to assess the stability of the results and investigated the effect of each study on pooled HR. We evaluated the publication bias and attached the corresponding

Begg's funnel plot. $P<0.05$ indicated a bias of study.[15] Correspondingly, we also carried out similar statistical analysis in different subgroups included country category, sample source, test method, sample size, miR-221/222 component, and cancer type.

Results

Summary of included studies

Thirty-two studies consisted of 2,693 samples satisfied the eligible studies[4–6,8–10,16–41] (Figure 1). Table 1 listed the main features of those available studies. Among those studies, Amankwah et al's[27] study involved in tumor samples of 37 non-recurrent and 28 recurrent PCa patients after radical prostatectomy aimed to explore whether the expression levels of miR-221 and miR-222 were related to PCa recurrence. Gyöngyösi et al[29] analyzed the association between the various miRNA of HCC and survival rate of patients treated with sorafenib after fine-needle aspiration biopsy. Mao et al[33] compared 2 different cohorts of regional lymph node involvement and no regional lymph node involvement to explore the prognostic potential of miR-222 expression in NSCLC. Cai et al[34] evaluated the prognostic value of miR-221 from 182 colon cancer (CC) patients after surgical resection both in the squamous cell carcinoma and adenocarcinoma cohorts. Finally, we integrated 32 studies independently into meta-analysis and established in Table 1.

There are 31 studies published in English and 1 study written in Chinese in current study. The maximum sample size was 180 and the minimum was 20. Tumor types were as follows: 1 triple-negative BC, 1 osteosarcoma, 1 PC, 4 gastric cancer (GC), 1 NK/T-cell lymphoma, 1 glioma, 4 NSCLC, 1 cutaneous malignant melanoma, 1 acute lymphoblastic leukemia, 2 colorectal cancer, 1 CC, 1 BC, 8 HCC, 1 glioblastoma (GBM), and 4 PCa. Besides, 1 ISH and 31 RT-PCR were applied in this study. Meanwhile, there were 8 FFPE; 7 blood; and 17 tissue. As for the survival outcomes, we split 32 eligible studies into 50 datasets: 27 for OS, 13 for DFS, 6 for PFS, and 4 for RFS. However, the cutoff value of included studies was inconsistent partly (Table 1).

Meta-analysis in OS

Twelve studies were included in univariate analysis to evaluate the prognostic value of overexpression of miR-221/222 cluster in human malignancy. Highly expressed miR-221/222

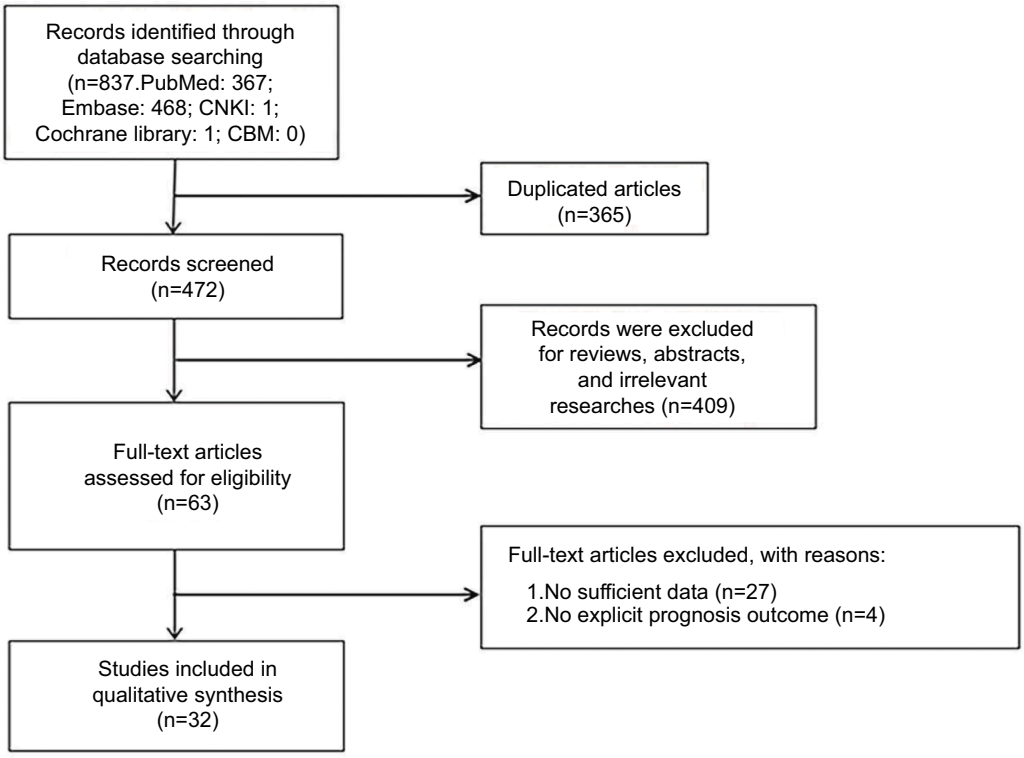

Figure 1 Flow diagram of the study selection process in the meta-analysis.
Abbreviations: CBM, Chinese Biomedical Literature Database; CNKI, Chinese National Knowledge Infrastructure.

Table 1 The main characteristics and survival data of included 32 studies

First author	Year	Country	Test method	Cancer type	miRNA	Sample source	Outcome	HR (95% CI)	Sample size	Cutoff value
Gramantieri L[4]	2009	Italy	qRT-PCR	HCC	miR-221	Tissue	OS/DFS	(U)1.12 (0.81–1.56)/ (U)1.56 (1.20–2.03)	51	Median
Greither T[5]	2010	Germany	qRT-PCR	PC	miR-222	Tissue	OS	(M)2.05 (1.05–4.00)	56	Median
Schaefer A[8]	2010	Germany	qRT-PCR	PCa	miR-221/ miR-222	Tissue	DFS	(U)0.93 (0.30–2.89)/ (U)0.69 (0.22–2.17)	76	Median
Wong QW[16]	2010	China	qRT-PCR	HCC	miR-222	Tissue	OS/DFS	(U)2.73 (1.41–5.30)/ (U)2.18 (1.20–3.98)	76	Median
Wang Y[17]	2010	China	qRT-PCR	ALL	miR-221	Tissue	OS	(U)0.54 (0.30–0.97)	32	Median
Spahn M[18]	2010	Germany	qRT-PCR	PCa	miR-221	FFPE	DFS	(M) 0.53 (0.29–0.95)	92	Median
Pu XX[19]	2010	China	qRT-PCR	CRC	miR-221	Blood	OS	(M) 3.48 (1.04–11.65)	103	Youden index
Guo HQ[20]	2010	China	qRT-PCR	N/T L	miR-221	Blood	OS	(U) 0.40 (0.17–0.95)/ (M) 0.18 (0.06–0.56)	79	Youden index
Wang RM[21]	2011	China	qRT-PCR	GC	miR-221	Tissue	OS	(U) 5.85 (2.32–14.72)	96	Median
Li JP[9]	2011	China	qRT-PCR	HCC	miR-221	Blood	OS	(M) 1.90 (1.24–2.98)	46	Average fold change
Yoon SO[22]	2011	Korea	qRT-PCR	HCC	miR-221	FFPE	DFS	(M) 3.07 (1.56–6.07)	115	Fold change =1
Karakatsanis A[23]	2011	Greece	qRT-PCR	HCC	miR-221	FFPE	OS	(M) 1.79 (1.24–2.58)	60	Average fold change
Liu K[24]	2012	China	qRT-PCR	GC	miR-221	Tissue	OS	(M) 2.32 (1.11–4.85)	92	Mean
Zhang C[25]	2012	China	ISH	GM	miR-221/ miR-222	Tissue	OS	(U) 2.03 (1.18–3.50)/ (U) 2.56 (1.16–5.65)	50	Final score =3
Rong M[26]	2013	China	qRT-PCR	HCC	miR-221	FFPE	DFS	(U) 1.40 (0.91–2.15)	48	Median
Amankwah EK[27]	2013	USA	qRT-PCR	PCa	miR-221	FFPE	DFS	(U) 1.79 (0.67–4.77)	65	Median
Kim BH[28]	2013	Korea	qRT-PCR	GC	miR-221	FFPE	OS	(M) 1.50 (0.70–2.90)	91	Fold change =3
Gyöngyösi B[29]	2014	Italy	qRT-PCR	HCC	miR-221	FFPE	OS/PFS	(U) 1.92 (0.61–6.10)/ (U) 1.32 (0.47–3.66)	20	Median
					miR-222		OS/PFS	(U) 2.04 (0.64–6.46)/ (U) 1.43 (0.51–3.99)		
Tao K[30]	2014	China	qRT-PCR	CRC	miR-221	FFPE	OS	(U) 2.42 (1.31–4.45)/ (M) 2.04 (1.10–3.81)	90	Median
Fu ZC[31]	2014	China	qRT-PCR	GC	miR-222	Blood	DFS	(U) 4.49 (2.68–7.49)/ (M) 3.41 (1.84–6.16)	114	2.23
Li P[32]	2014	China	qRT-PCR	CMM	miR-221	Blood	OS/DFS	(M) 3.19 (1.78–6.78)/ (M) 2.12 (1.96–8.55)	72	2.95
Mao KP[33]	2014	China	qRT-PCR	NSCLC	miR-222	Tissue	OS	(M) 3.31 (1.97–5.58)	100	Median
Cai K[34]	2015	China	qRT-PCR	CC	miR-221	Tissue	OS	(U) 2.19 (1.11–4.43)/ (M) 2.39 (1.21–4.91)	182	Median
Zhang YH[35]	2015	China	qRT-PCR	NSCLC	miR-221	Tissue	OS	(U) 1.87 (1.27–2.76)/ (M) 1.87 (1.27–2.77)	104	Mean
Yang Z[36]	2015	China	qRT-PCR	OSM	miR-221	Blood	OS/RFS	(M) 7.66 (1.83–15.92)/ (M) 6.82 (1.33–13.69)	108	Median
Goto Y[37]	2015	Japan	qRT-PCR	PCa	miR-222	Tissue	PFS	(U) 0.35 (0.14–0.92)/ (M) 0.21 (0.07–0.64)	92	NM
Eissa S[6]	2015	Egypt	qRT-PCR	BC	miR-221	Tissue	RFS	(M) 14.84 (1.80–9.50)	76	1.03
Liao L[38]	2016	China	qRT-PCR	NSCLC	miR-221/ miR-222	Tissue	PFS	(U) 2.52 (1.07–5.92)/ (U) 3.15 (1.30–7.65)	55	Median
Xie DF[39]	2017	China	qRT-PCR	HCC	miR-221	Tissue	OS	(M) 1.74 (1.00–3.77)	70	≥1.795
Liu Y[40]	2017	China	qRT-PCR	NSCLC	miR-221	Tissue	OS	(M) 2.58 (1.41–4.85)	151	Median
Deng L[10]	2017	China	qRT-PCR	TNBC	miR-221	Tissue	DFS	(U) 0.49 (0.27–0.88)/ (M) 0.48 (0.26–0.88)	125	NM
Zhao H[41]	2017	USA	qRT-PCR	GBM	miR-222	Blood	DFS	(U) 1.71 (1.07–3.63)	106	Median

Abbreviations: TNBC, triple-negative breast cancer; BC, breast cancer; NSCLC, non-small cell lung cancer; HCC, hepatocellular carcinoma; GC, gastric cancer; CRC, colorectal cancer; ALL, acute lymphoblastic leukemia; CMM, cutaneous malignant melanoma; CC, colon cancer; N/T L, NK/T-cell lymphoma; GBM, glioblastoma; GM, glioma; OSM, osteosarcoma; PC, pancreatic cancer; PCa, prostate cancer; NM, not mentioned; qRT-PCR, quantitative reverse transcription-polymerase chain reaction; OS, overall survival; DFS, disease-free survival; ISH, in situ hybridization; PFS, progression-free survival; RFS, relapse-free survival.

cluster of tumors was connected with poor OS (HR =1.69, 95% CI: 1.18–2.44, *P*<0.01) (Figure 2A). In addition, subanalyses results revealed that there were certain correlations (Table 2). 15 studies were involved in multivariate analysis to conduct an evaluation regarding the prognostic value of miR-221/222 cluster. Meanwhile, tumor-associated miR-221/222 cluster overexpression also connected with poor OS (HR =2.10, 95% CI: 1.63–2.69, *P*<0.01) (Figure 2B). Similarly, several subgroups have an analogous result (Table 2).

Meta-analysis in DFS/PFS/RFS

There were 9, 5, and zero studies about DFS/PFS/RFS involved in univariate analysis. Correspondingly, 5, 1, and 4 studies about DFS/PFS/RFS were collected in multivariate analysis, respectively. Ultimately, we detected no association between highly expressed miR-221/222 cluster with DFS (univariate: HR =1.45, 95% CI: 0.94–2.25, *P*=0.10; multivariate: HR =1.40, 95% CI: 0.59–3.34, *P*=0.45) (Figure 3), PFS (univariate: HR =1.41, 95% CI: 0.65–3.05, *P*=0.38; multivariate: HR =0.21, 95% CI: 0.07–0.63, *P*=0.006) (Figure S1), and RFS (multivariate: HR =2.18, 95% CI:0.34–13.89, *P*=0.41) (Figure S2).

Sensitivity analysis

We removed each single study respectively to evaluate its specific effect on the mixed HRs, and sensitivity analysis indicated a relatively stable mixed result. Univariate and multivariate analyses of the pooled results (OS and DFS) were performed in Figure 4A, B and Figure S3A, B, but the result of PFS and RFS only in single-variate analysis was not shown.

Publication bias

According to Begg's funnel plot, there was no publication bias of OS (*P*=0.784>0.05) (Figure 5A), DFS (*P*=0.322>0.05),

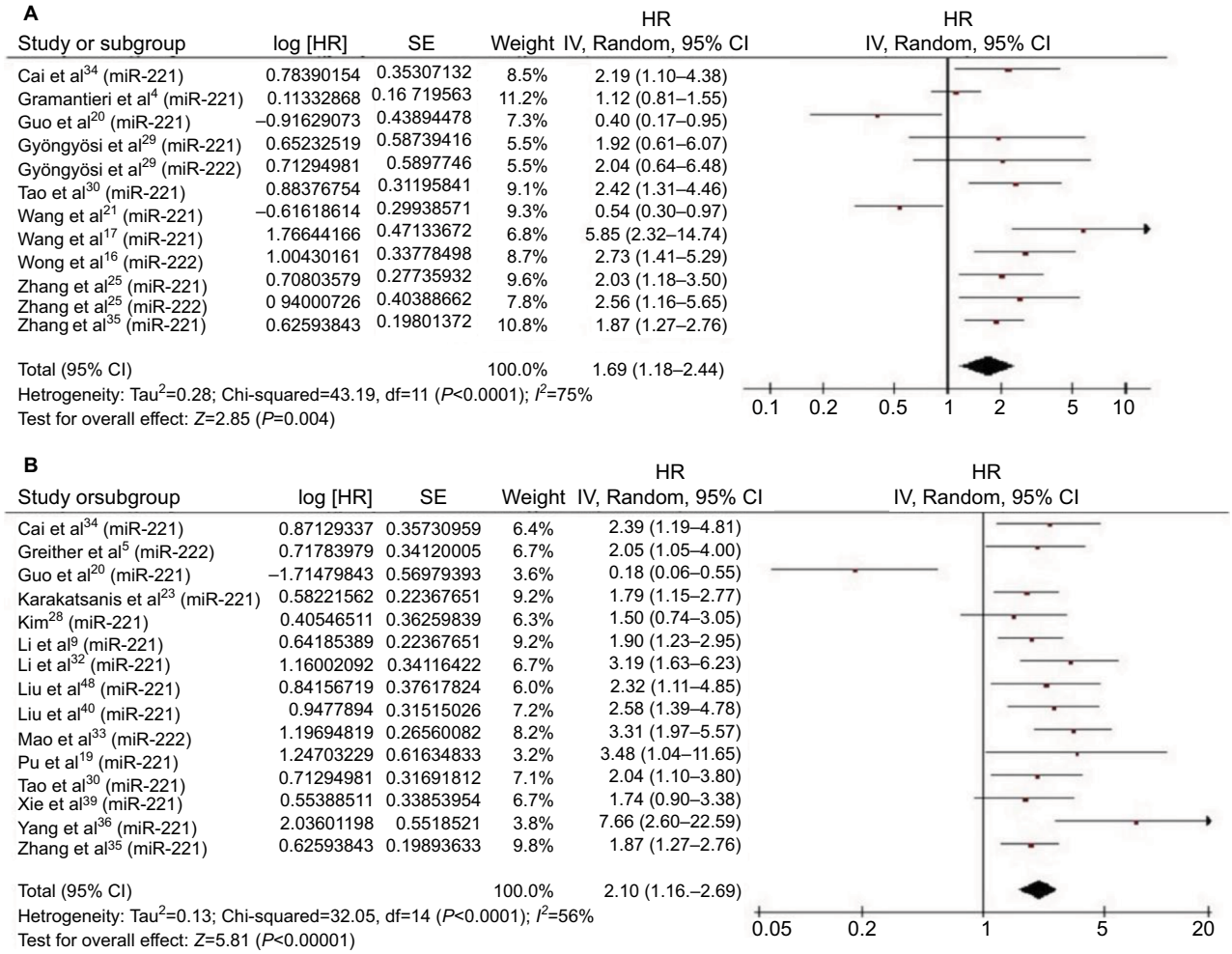

Figure 2 Forest plot of the association between high expression of miR-221/222 family in various tumors and OS under different types of analysis.
Note: (**A**) univariate analysis; (**B**) multivariate analysis.
Abbreviations: HR, hazard ratio; OS, overall survival; SE, standard error.

Table 2 Stratified analysis of the high expression of miR-221/222 family and overall survival

Categories	Subgroups	Univariate analyses					multivariate analyses				
		No of dataset	HR (95% CI)	*P*-value	I^2	Ph	No of dataset	HR (95% CI)	*P*-value	I^2	Ph
All		12	**1.26** (1.07–1.47)	0.004	75%	0.000	15	**2.10** (1.63–2.69)	0.000	56%	0.004
Country	China	9	**1.28** (1.05–1.56)	0.02	79%	0.000	12	**2.19** (1.60–3.01)	0.000	64%	0.001
	Others	3	1.09 (0.95–1.24)	0.21	0%	0.45	3	**1.28** (1.12–1.48)	0.000	0%	0.82
Test method	qRT-PCR	10	**1.23** (1.02–1.48)	0.03	78%	0.000	15	**2.10** (1.63–2.69)	0.000	56%	0.004
	ISH	2	**1.40** (1.16–1.71)	0.000	0%	0.64	/	/	/	/	/
Sample source	FFPE	3	**1.42** (1.15–1.76)	0.001	0%	0.93	3	**1.29** (1.12–1.48)	0.000	0%	0.82
	Tissue	8	**1.30** (1.08–1.58)	0.005	78%	0.000	7	**1.37** (1.24–1.52)	0.000	0%	0.94
	Blood	1	**0.67** (0.46–0.98)	0.04	/	/	5	1.35 (0.90–2.03)	0.15	85%	0.000
miR221/222	miR221	9	1.20 (1.00–1.45)	0.05	79%	0.000	13	**2.01** (1.52–2.65)	0.000	58%	0.004
Component	miR222	3	**1.50** (1.23–1.84)	0.000	0%	0.91	2	**2.73** (1.72–4.33)	0.000	19%	0.27
Sample size	≥100	2	**1.33** (1.15–1.55)	0.000	0%	0.70	6	**2.72** (1.96–3.79)	0.000	35%	0.18
	<100	10	**1.24** (1.02–1.51)	0.03	78%	0.000	9	**1.27** (1.10–1.47)	0.001	60%	0.01
Cancer type	NSCLC	1	**1.87** (1.27–2.76)	0.002	/	/	3	**2.42** (1.69–3.45)	0.000	35%	0.22
	HCC	4	1.71 (1.00–2.90)	0.05	53%	0.09	3	**1.83** (1.38–2.42)	0.000	0%	0.97
	others	7	1.64 (0.87–3.10)	0.13	83%	0.000	9	**2.05** (1.26–3.33)	0.004	71%	0.000

Note: The bold font indicated *P*-value of the HR was <0.05.

Abbreviations: NSCLC, non-small cell lung cancer; HCC, hepatocellular carcinoma; qRT-PCR, quantitative reverse transcription-polymerase chain reaction; ISH, in situ hybridization; FFPE, formalin-fixed and paraffin-embedded; HR, hazard ratio; CI, confidence interval; P^h, *P*-value of heterogeneity test.

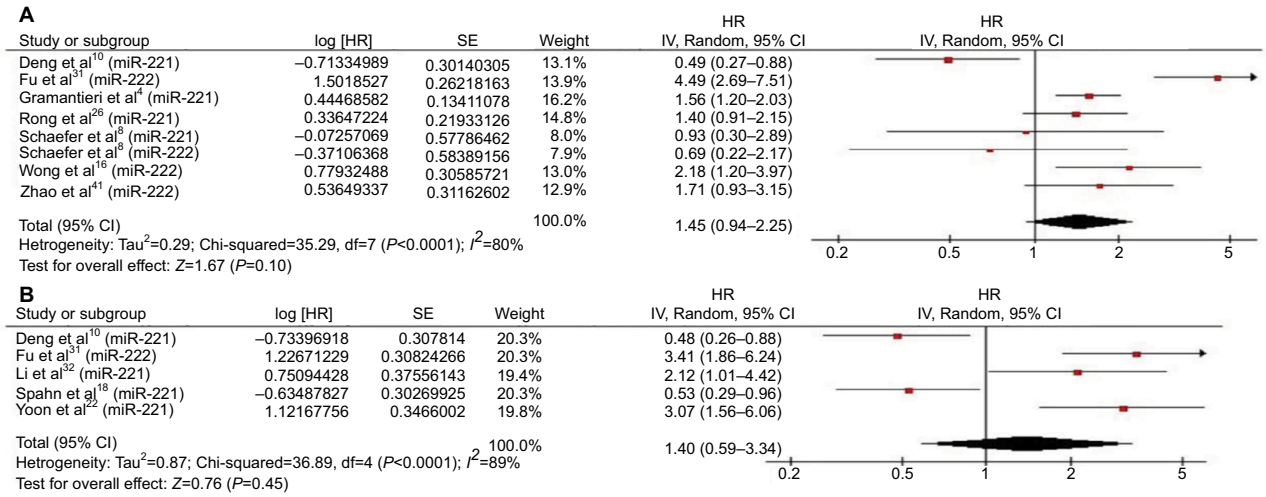

Figure 3 Forest plot of the association between high expression of miR-221/222 family in various tumors and DFS under different types of analysis.

Note: **(A)** univariate analysis; **(B)** multivariate analysis.

Abbreviations: DFS, disease-free survival; HR, hazard ratio; SE, standard error.

and PFS (*P*=0.624>0.05) among univariate analysis, and no publication bias of OS (*P*=0.324>0.05) (Figure 5B), DFS (*P*=0.624>0.05), and RFS (*P*=0.497>0.05) among multivariate analysis. Similar to that, we also performed evaluations of subgroup of OS. There was no evidence for publication bias in any subgroup. However, the publication bias of PFS or RFS was not evaluated due to the fewer or no datasets for meta-analysis.

Discussion

Currently, exploring the clinically available cancer signatures is still the hotspot of researches due to the complexity of tumor. Dysregulations of distinct miRNA fingerprints often occur in specific types of human malignant tumors and obviously associated with cancer diagnosis, therapy, and even prognosis.[42,43] Numerous studies have demonstrated that miR-221/222 cluster plays a significantly regulatory role in the prognosis of several types of tumors. For instance, miR-222 overexpression promoted HCC cell migratory through activating AKT phosphorylation assisted by the regulation of protein phosphatase 2A subunit B, contributing to both the development and poor prognosis of HCC.[16] Besides, it is reported that miR-221 negatively regulates poly ADP-Ribose

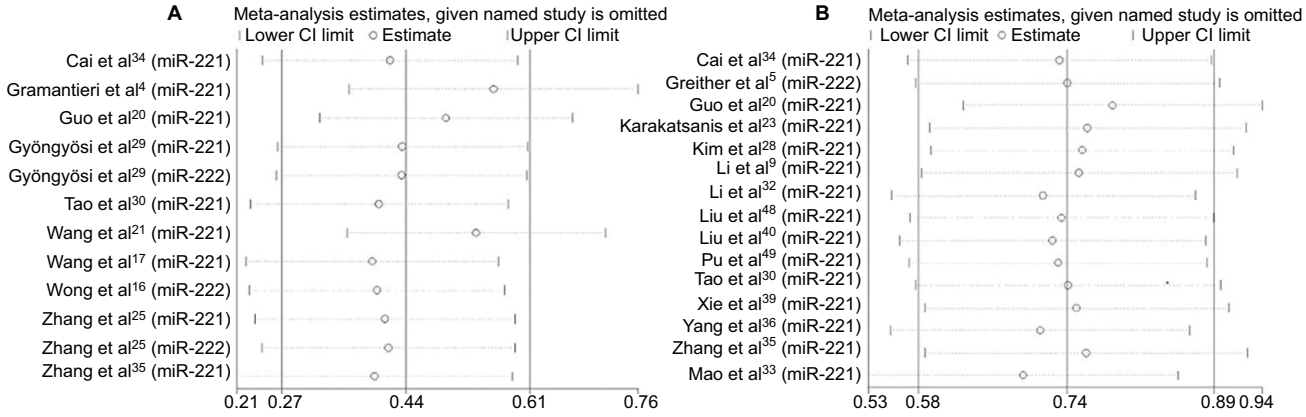

Figure 4 One-way sensitivity analysis of high expression of miR-221/222 family in various tumors with OS under different types of analysis.
Note: (**A**) univariate analysis; (**B**) multivariate analysis.
Abbreviation: OS, overall survival.

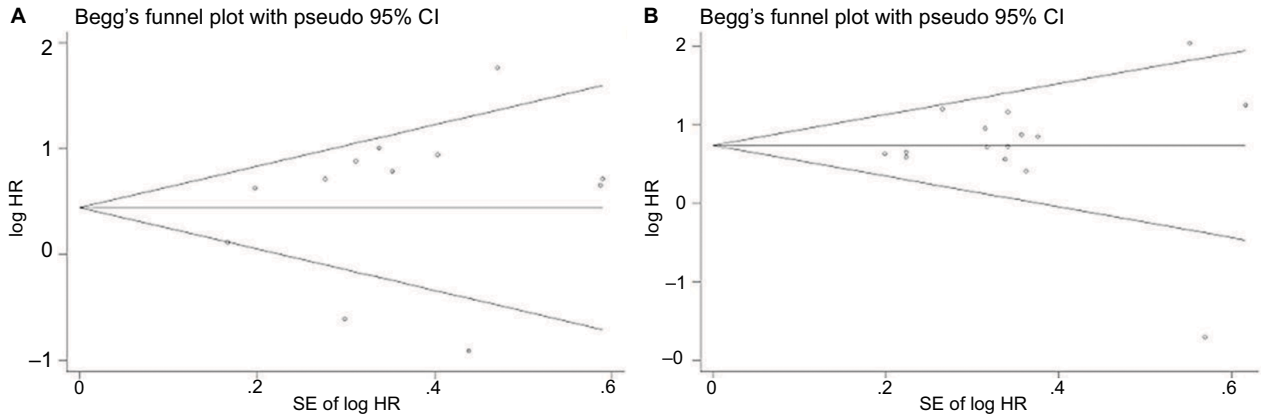

Figure 5 Funnel plot analysis of publication bias of high expression of miR-221/222 family in various tumors with OS under different types of analysis.
Notes: (**A**) univariate analysis; (**B**) multivariate analysis.
Abbreviations: HR, hazard ratio; OS, overall survival.

polymerase 1 expression levels by 3′-untranslated region binding, thereby affecting TNBC patients' prognoses.[10] Meanwhile, overexpression of miRNA-221 enhanced cell proliferation via downregulating the target gene apoptotic protease activating factor-1 (APAF-1), while APAF-1 expression was associated with a poor prognosis.[44] It is reported that the upregulation of miR-221/222 cluster in GBM may be a key factor in the decrease of p27Kip1 expression levels and as such, will correlate with adverse prognosis.[45] Similarly, miRNA-222 could target HIPK2 to promote GC cell proliferation, invasion, and inhibited apoptosis, revealing a poor survival outcome for GC patients.[46] Moreover, the expression of miR-221 exhibited modulatory effect on BIM-Bax/Bak axis, upregulated target gene BIM, induced by miR-221 knockdown, can promote cisplatin-eliciting apoptosis through inducing mitochondrial dysfunction, revealing a poor survival outcome for BC patients.[47] Hence, we proposed that activation or inhibition of multiple pathways might play synergistic roles in the impact of miRNA-221/222 cluster expression on prognosis. However, the clinically prognostic value of dysregulated miR-221/222 expression often affected by small sample-size studies with insufficient data and thus inconsistent and unconvincing.

Meta-analysis is a useful tool, which can get a relatively precise result and provide convincing evidence through integrating and assessing inconsistent outcomes from different studies. We have explored the potential associations in overall population and the corresponding subgroups via combining univariate and multivariate analyses. Likewise, several subgroups have similar statistical results. However, no association of miR-221/222 family was detected with DFS/PFS/RFS.

Generally, we only explored the potential associations in corresponding subgroups of OS due to the sufficient data. When stratified by sample source, given the result in multivariate analysis, blood miR-221/222 expression was unlikely as a

promising marker on tumor prognosis. Meanwhile, the result was also not convincing in univariate analysis based on only one study. However, the miR-221/222 expression in FFPE or tissue might be associated with poor OS of carcinoma. When stratified by the sample size, we discovered that high expression of miR-221/222 cluster connected with unfavorable OS significantly in both groups with different sample sizes. When stratified by miRNA, the result indicated that the over expression of miR-221 and miR-222 both can predict poor OS apparently in multivariate analysis. However, we proposed overexpression miRNA-221/222 cluster could be viewed as a protection factor in PCa after integrating 4 studies on PCa[48] (Figure S4). It is supposed that overexpression miRNA-221/222 cluster could repress PCa cell invasion[37] and decrease prostate-specific antigen expression mediated by androgen receptor,[49] which may participate in the protection process. Besides, lack of sufficient data indeed has a certain impact on the intriguing result. Future studies from multicenter comprising larger cohort size are needed to verify the current findings.

Actually, there were some limitations in our study that we should notice in the current meta-analysis. First, as for OS, part of the outcome failed to be analyzed separately due to the small sample size and no publication bias could be assessed in the current meta-analysis. In consideration of the limitations of qualified studies quantities, we did not process subgroup analysis for DFS/PFS/RFS. But the relative DFS/PFS/RFS data presented in this study may improve our understanding of cancer progression and relapse. Moreover, the design of studies, cutoff values, and measure methods being distinct in different studies, we could not consolidate those inconsistent objective elements, which were influential factors to the results. In addition, we did not compare the difference of ethnicities because of the limited number of different countries or races.

Conclusion

A significant correlation was explored in overall population and corresponding subgroups. Concretely, it presented that miR-221/222 family overexpression was significantly linked with poor OS, while no relationship was found between higher miR-221/222 family expression and DFS/PFS/RFS. Besides, sample sizes had no effect on our results, majority of subgroup analysis result was consistent with overall conclusion. Besides, miRNA-221/222 cluster from tissue or FFPE was more convincing in prediction of OS of tumor patients compared with blood-derived miRNA-221/222 cluster. Moreover, miRNA-221/222 cluster overexpression could be viewed as a protection factor in PCa to some extent. The elucidation of deregulated miR-221/222 cluster is expected to improve the understanding on tumor and promote the further development of biomarkers in cancer prognosis.

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Disclosure

The authors report no conflicts of interest in this work.

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Supplementary materials

Figure S1 Forest plot of the association between high expression of miR-221/222 family in various tumors and PFS under different types of analysis. (**A**: univariate analysis; **B**: multivariate analysis).
Abbreviations: HR, hazard ratio; PFS, progression-free survival; SE, standard error.

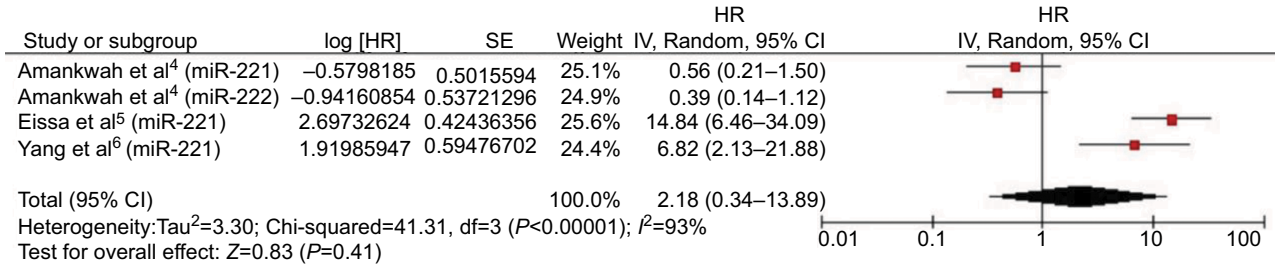

Figure S2 Forest plot of the association between high expression of miR-221/222 family in various tumors and RFS under multivariate analysis.
Abbreviations: HR, hazard ratio; RFS, relapse free survival; SE, standard error.

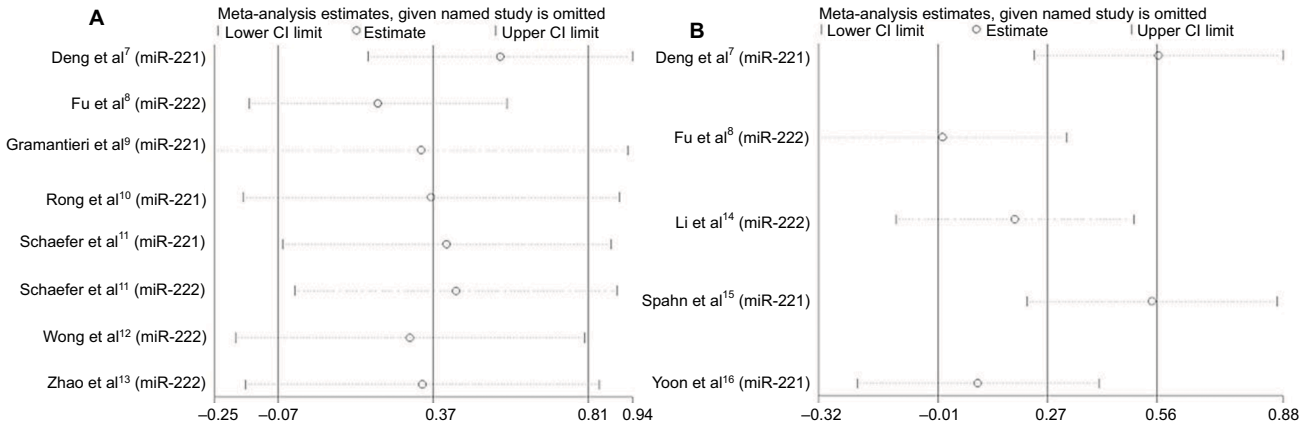

Figure S3 One-way sensitivity analysis of high expression of miR-221/222 family in various tumors with DFS under different types of analysis.
Note: (**A**) univariate analysis; (**B**) multivariate analysis.
Abbreviation: DFS, disease-free survival.

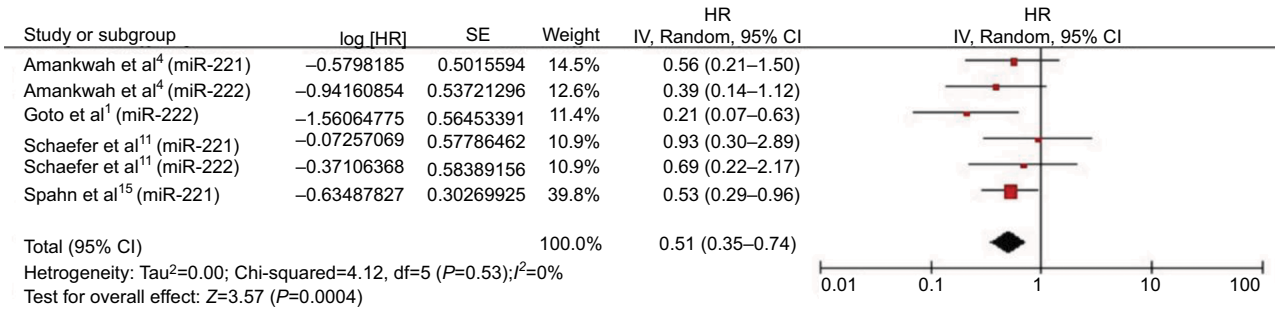

Figure S4 Forest plot of the association between high expression of miR-221/222 family in prostate cancer and DFS/RFS.
Abbreviations: HR, hazard ratio; DFS, disease-free survival; RFS, relapse free survival; SE, standard error.

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