

## REVIEW ARTICLE

# Prognostic Value of miR-222 in Various Cancers: a Systematic Review and Meta-Analysis

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### SUMMARY

**Background:** MicroRNAs (miRNAs) play important roles in cancer development. MiR-222, which is deregulated in multiple types of cancers, shows potential as a prognostic biomarker; however, the association between miR-222 expression and cancer prognosis was controversial in previous studies. Here we analyzed the relationship between miR-222 and the survival of cancer patients.

**Methods:** A systematic search of PubMed, Web of Science, Ovid, SciFinder, Cochrane Library, Wan Fang, and the Chinese National Knowledge Infrastructure databases up until June 8, 2015 was performed to clarify the prognostic value of miR-222 in cancers. The hazard ratio (HR) and 95% confidence interval (95% CI) were extracted to evaluate the effect.

**Results:** Sixteen published articles involving 1,354 patients were included in this meta-analysis. High expression of miR-222 was associated with poor overall survival with a pooled HR of 1.86 (95% CI, 1.57 - 2.14), without significant heterogeneity, and no publication bias was detected.

**Conclusions:** Our results showed that high expression of miR-222 is associated with poor overall survival of patients with cancers and could be used as a predictive biomarker for prognosis of various cancers.

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#### KEY WORDS

miR-222, prognosis, meta-analysis, cancer

#### INTRODUCTION

The World Health Organization has reported that nearly 14.1 million new cases of cancer occurred globally and caused ~8.2 million or 14.6% of all human deaths in 2012 [1]. Although different therapies have been used to treat malignancies, the 5-year survival rate is still unsatisfactory. Moreover, the patients at the same tumor clinical stage have different outcomes, which calls for novel biomarkers to predict survival and guide the best choice of clinical treatment.

MicroRNAs (miRNAs) are a specific class of noncoding RNAs, 20 - 22 nucleotides in length. The binding to the 3' untranslated region (3' UTR) of target mRNAs through base-pairing results in degradation or transcriptional inhibition and possible negative regulation of

gene expression [2]. Recently, many studies have confirmed that miRNAs such as miR-21 and miR-375, whether upregulated or downregulated, play important roles in cancer development and can be used as novel targets to evaluate prognosis and guide cancer therapy [3-5]. MiR-222, a member of the miR-221/222 family encoded on the X chromosome P11.3 region, is highly conserved in vertebrates. Through regulating certain target genes, it participates in the development of different kinds of disorders. For example, miR-222 is validated to increase endothelial cell proliferation and migration resulting in immature and leaky intraplaque neovascularization during atherosclerosis [6]. Similarly, it has also been verified to promote microtubule formation and angiogenesis through modulating c-Kit, a tyrosine kinase cytokine receptor expressed in mature endothelial cells [7]. In addition, miR-222 inhibits cell apoptosis and prolongs the survival of rat dorsal root ganglion neurons after peripheral nerve injury by suppressing tissue inhibitor of metalloproteinase 3 [8]. It has been reported that miR-222 is upregulated in different types of malignant tumors such as breast cancer, glioma, and hepatocellular carcinoma [9-11]. By targeting specific genes, deregulated miR-222 can promote cell growth and inhibit apoptosis as well as being involved in tumor cell metastasis and invasion. Some studies have shown that upregulated miR-222 in tumor tissue or cells tends to be associated with a poor prognosis [12-14], but inconsistencies exist. For instance, by downregulating matrix metalloproteinase expression, miR-222 inhibits oral tongue squamous carcinoma cell invasion, suggesting that miR-222 might have a protective role against cancer development [15]. In order to clarify the actual value of miR-222 in cancer prognosis, we performed this meta-analysis.

## MATERIALS AND METHODS

### Search strategy

This study was performed according to the standard guidelines for meta-analysis. Two investigators (T Wei and P Ye) independently carried out a comprehensive literature search for original articles up to June 8, 2015 involving miR-222 and prognosis of cancer. Both Medical Subject Heading (Mesh) terms and free text words were used to acquire relevant studies by searching PubMed, Web of Science, Ovid, SciFinder, Cochrane Library, Wan Fang, and Chinese National Knowledge Infrastructure (CNKI). There was no language restriction. The search terms were: “microRNA-222”, “miR-222”, “neoplasm”, “cancer”, “malignant tumor”, “carcinoma”, “prognosis”, “prognostic”, “survival”, “outcome”, and “metastasis”.

### Study selection

Articles that satisfied the following criteria were included: (a) studies involving any type of cancer; (b) miR-222 expression was measured; (c) the relationship be-

tween miR-222 and clinical outcomes was investigated; and (d) Hazard Ratios (HRs) and 95% confidence intervals (95% CIs) were provided, or there was sufficient information to estimate them. If a study was absent of key information to carry on further analysis, it was excluded. Meanwhile, if replicated patient cohort was published in different articles, only the most recent or complete one was included. Animal experiments, single case report, letters, and reviews were excluded. The disagreements were resolved by consensus.

### Data extraction

Based on the authentic checklist of Meta-analysis of Observational Studies in Epidemiology (MOOSE) [16], two investigators (T. Wei and P. Ye) carefully and independently evaluated the available articles and extracted the data. For each study, the following items were collected: first author's name, year of publication, country, patient ethnicity, tumor type, sample type, testing methods, number of patients, age, gender, clinical stage, cut-off values, follow-up, treatment data, calculation methods, overall survival (OS), disease-free survival (DFS), and metastasis-free survival (MFS).

### Quality assessment

Two investigators (T. Wei and P. Ye) evaluated the study methodology separately and scored them based on the scale reported previously [17]. Briefly, accepted studies were assessed through four aspects: the scientific design, the description of the methods to verify the abnormal expression of miR-222, the generalizability of the results, and the analysis of data. Each classification had a maximum score of 10 points. Therefore, the total score was 40 points. When an item did not conform to a study, the corresponding value was not taken into account in the total of relevant category. The final score was presented as percentages with the range from 0 to 100% and higher score denoted a better quality.

### Statistical analysis

We extracted the data using three main methods. The ideal and most accurate way was to retrieve the reported HRs of miR-222 for survival combined with 95% CIs and p-values directly from the original articles. For studies that did not provide the HRs or 95% CIs, we estimated them using the available information by calculating from the number of events, patients at risk, or p-values. However, a few studies presented the results in the form of Kaplan-Meier curves; consequently, we reconstructed HR estimates following Tierney et al. by assuming that the censored patient rate was constant during follow-up [18]. Engauge Digitizer (version 4.0) was used to extract the survival rate at specific time points to reduce errors. All these data were input in the form produced by Tierney et al., and approximate curves were generated [18]. Through several adjustments of the original plots, we obtained curves that had the closest similarity with the published plot and estimated the HR with 95% CIs from them. If necessary, we acquired the origi-

**Table 1. Characteristics of all the studies included in the meta-analysis.**

Study	Country	Ethnicity	Tumor Type	Sample size	Age (years, n)	Gender (M/F)	Sample	Clinical Stage	Method	Cutoff point	Outcome measured	Survival analysis	Estimation method *	Quality score (%)
Schaefer et al. 2009	Germany	Caucasian	Prostate cancer	76	Median 63 (49 - 74)	NR	Frozen tissue	I - IV	qRT-PCR	T/N ratio > 1.5	DFS	Univariate	2	64.2
Greither et al. 2009	Germany	Caucasian	Pancreatic cancer	56	Mean 61.7 (34 - 80)	34/22	Frozen tissue	I - IV	qRT-PCR	T/N ratio > 2.36	OS	Multivariate	1	60.1
Wong et al. 2010	China	Asian	Hepatocellular carcinoma	76	Median 57 (48 - 66.5)	NR	Frozen tissue	T1, T2, T3	qRT-PCR	T/N ratio > 2	DFS, OS	Univariate	2	57.7
Delfino et al. 2011	USA	Caucasian	Glioblastoma	253	NR	162/91	Frozen tissue	NR	Microarray	NR	OS	Multivariate	1	60.8
Zhang et al. 2012	China	Asian	Glioma	36	NR	NR	FFPE	High grade	In situ Hybridization	Score > 3	OS	Univariate	2	70.2
Puerta-Gil et al. 2012	Spain	Caucasian	Bladder cancer	113	Median 73 (35 - 94)	101/12	FFPE	Low/high grade	qRT-PCR	NR	OS	K - M	3	58.2
Schultz et al. 2012	Denmark	Caucasian	Pancreatic cancer	138	Median 64 (31 - 85)	NR	FFPE	I - II	Microarray	10 percentile vs. 90 percentile miRNA expression	OS	Multivariate	1	93.5
Lee et al. 2013	China	Asian	Pancreatic cancer	60	≤ 60, 18; > 60, 42	38/22	Frozen tissue	I - IV	qRT-PCR	Median	OS	Multivariate	1	72.3
Kim et al. 2013	Korea	Asian	Gastric cancer	91	Median 61 (25 - 80)	57/34	FFPE	I - IV	qRT-PCR	T/N ratio > 3	MFS, OS	Multivariate	1	94.3
Zhan et al. 2013	China	Asian	Hepatocellular carcinoma	49	Mean 55 (36 - 65)	38/11	Serum	I - IV	qRT-PCR	Median	OS	Multivariate	1	67.1
Xu et al. 2013	China	Asian	Hepatocellular carcinoma	76	< 50, 35; ≥ 50, 41	47/29	Serum	I - III	qRT-PCR	Mean	OS	K - M	3	61.9
Zhang et al. 2014	China	Asian	Bladder cancer	97	< 60, 59; ≥ 60, 38	51/46	Frozen tissue	Ta - T2, G1/2, G3	qRT-PCR	T/N ratio > 5.15	OS	Multivariate	1	93.5
Mao et al. 2014	China	Asian	Non-small cell lung cancer	100	< 60, 34; ≥ 60, 66	59/41	Frozen tissue	I - IV	qRT-PCR	Median	OS	Multivariate	1	80.1
Fu et al. 2014	China	Asian	Gastric cancer	114	≤ 50, 68; > 50, 46	54/60	Plasma	I - IV	qRT-PCR	T/N ratio > 2.23	DFS, OS	Multivariate	1	82.4
Li et al. 2014	China	Asian	Glioma	45	< 50, 18; ≥ 50, 27	30/15	Frozen tissue	I - IV	qRT-PCR	Mean	OS	K - M	1	93.5
Zhang et al. 2015	China	Asian	Glioma	50	Mean 51.2	29/21	Plasma	I - IV	qRT-PCR	T/N ratio > 2.67	OS	K - M	1	85.2

Abbreviations: FFPE - formalin-fixed and paraffin-embedded, OS - overall survival, DFS - disease-free survival, MFS - metastasis-free survival, NR - not reported. \* 1 Hazard Ratios directly from articles; 2 Hazard Ratios estimated from the total number of events and p-values; 3 Hazard Ratios extracted from Kaplan-Meier curves.

**Table 2. The aberrant expression and target genes or pathways of miR-222 in different malignancies.**

Type of malignancy	miR-222 expression	Validated target genes or pathways	Reference
Breast cancer	Up	AdipoR1, Dicer, SOCS1, CDKN1B	[9,33,35]
Gastric cancer	Up	RECK, PTEN	[36,37]
Hepatocellular carcinoma	Up	GNAI3, PPP2R2A	[24,38]
Glioblastoma	Up	PUMA, PTP $\mu$	[32,39]
Glioma	Up	DKK2, Wnt/ $\beta$ -catenin pathway	[40]
Thyroid cancer	Up	p27Kip1	[33]
Pancreatic cancer	Up	NR	[22,23]
Bladder cancer	Up or Down	PI3K/Akt pathway	[13,41,47]
Prostate cancer	Up or Down	SIRT1, p27Kip1	[42,43]

Abbreviations: AdipoR1 - adiponectin receptor 1, SOCS1 - suppressor of cytokine signaling 1, CDKN1B - cyclin-dependent kinase inhibitor 1B, RECK - reversion-inducing cysteine-rich protein, PTEN - phosphatase and tensin homolog, GNAI3 - guanine nucleotide binding protein (G protein), alpha inhibiting activity polypeptide 3, PPP2R2A - protein phosphatase 2, regulatory subunit B, alpha; PUMA, p53 upregulated modulator of apoptosis, PTP $\mu$  - protein tyrosine phosphatase  $\mu$ , DKK2 - dickkopf homolog 2, SIRT1 - sirtuin 1.

nal data directly from the authors.

The pooled HRs and 95% CIs were calculated using a fixed-effect model if the heterogeneity was not significant. If it was, then a random-effect model was used [19]. The Higgins  $I^2$  statistic was used to assess the heterogeneity with  $I^2 > 50\%$  suggesting significant heterogeneity. In general, a calculated HR  $> 1$  demonstrated a worse survival for the group with high miR-222 expression. Publication bias was evaluated using Begg's test [20]. Statistical analysis was performed using Stata software version 13.0 (Stata Corporation, College Station, TX, USA).  $P < 0.05$  was considered statistically significant.

## RESULTS

### Study characteristics

The comprehensive search of the databases gave 997 relevant articles. By viewing the titles and abstracts, 979 studies were excluded for being irrelevant or for repetition. After carefully reading the remaining 18 full-text articles, 2 were excluded for not providing enough information to estimate HRs and 95% CIs. Hence, 16 studies (from 2009 to 2015) were included in the present analysis [10-14,21-31] (Figure 1). A total of 1,354 patients were involved, ranging from 36 to 253 patients per study. Eight types of malignant tumor were investigated, among which digestive system malignancies had the greatest proportion (3 hepatocellular carcinoma, 2 gastric cancer, and 3 pancreatic cancer); the other types of malignancies were 3 glioma, 2 bladder cancer, 1 prostate cancer, 1 non-small cell lung cancer, and 1 glioblastoma. Samples included frozen or formalin-fixed and paraffin-embedded tissues and blood samples (plasma or serum). Cutoff values of miR-222 were different among studies. Fifteen of the studies explored the

prognostic value of miR-222 for OS, 3 for DFS, and 1 for MFS (Table 1).

A total of 19 HRs were obtained: 14 HRs were extracted directly from the original articles, 3 HRs were estimated by the total number of events and p-value, and 2 HRs were approximated using a K-M curve.

### Quality assessment

Sixteen studies included in our meta-analysis were assessed for quality according to the scale reported by Steels et al. After assessing through four aspects, each study was given a score to present its quality. The score of all the included studies varied from 57.7% to 94.3%, with a mean value of 74.7%. A higher score indicated a better quality, thus all the studies were included in the following analysis. The score of each study is listed in Table 1.

### Association between miR-222 and malignancies

#### Analysis for OS

Fifteen studies evaluated the relationship between miR-222 and OS [10-14,22-31], and the test for heterogeneity was not significant ( $I^2 = 36.3\%$ ,  $P_{\text{heterogeneity}} = 0.079$ ). Therefore, we used the fixed-effects model to calculate the pooled HR. The results showed that high expression of miR-222 predicted poor overall outcome with a pooled HR of 1.86 (95% CI, 1.57 - 2.14;  $p < 0.0001$ ; Figure 2). Subgroup analysis was not performed due to the indistinctive heterogeneity.

#### Analysis for DFS

Since 3 studies involving DFS showed obvious heterogeneity, we used a random effect model to calculate the pooled HR [12,21,24]. It was found that high expression of miR-222 did not reach significance in predicting a worse outcome, with a pooled HR of 1.97 (95% CI, 0.40 - 3.53;  $I^2 = 76\%$ ,  $P_{\text{heterogeneity}} = 0.016$ , random ef-

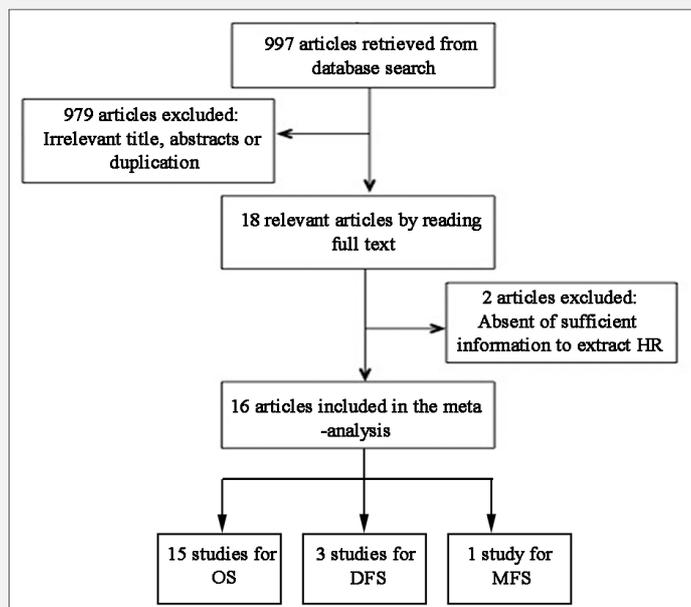


Figure 1. Flow diagram of the study selection.

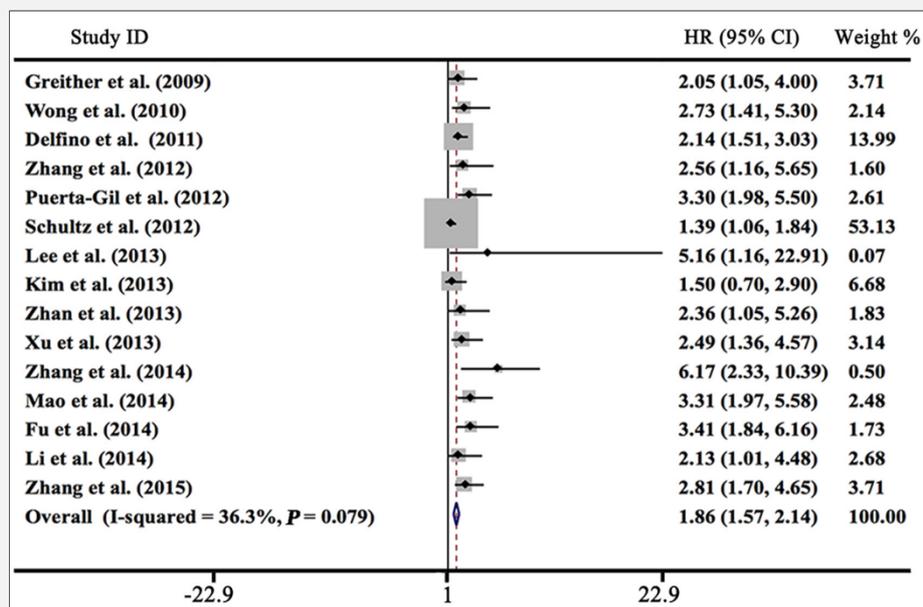


Figure 2. Forest plot of the studies evaluating the hazard ratios of high miR-222 expression compared to low miR-222 expression in OS for different types of cancer.

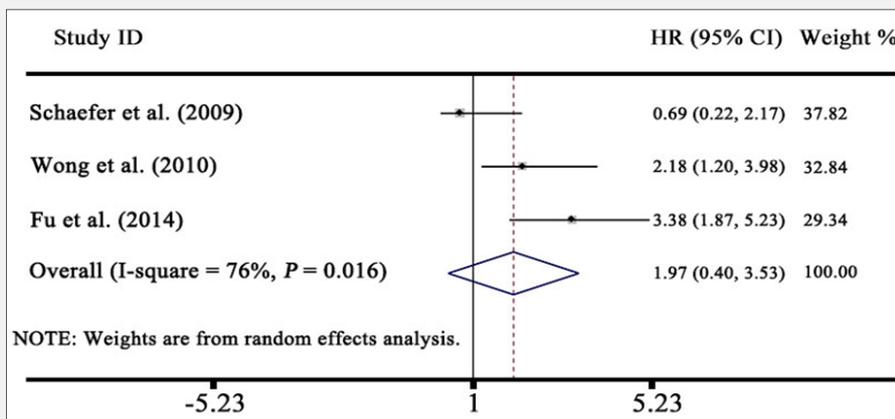


Figure 3. Forest plot of the studies evaluating the hazard ratios of high miR-222 expression compared to low miR-222 expression in DFS for different types of cancer.

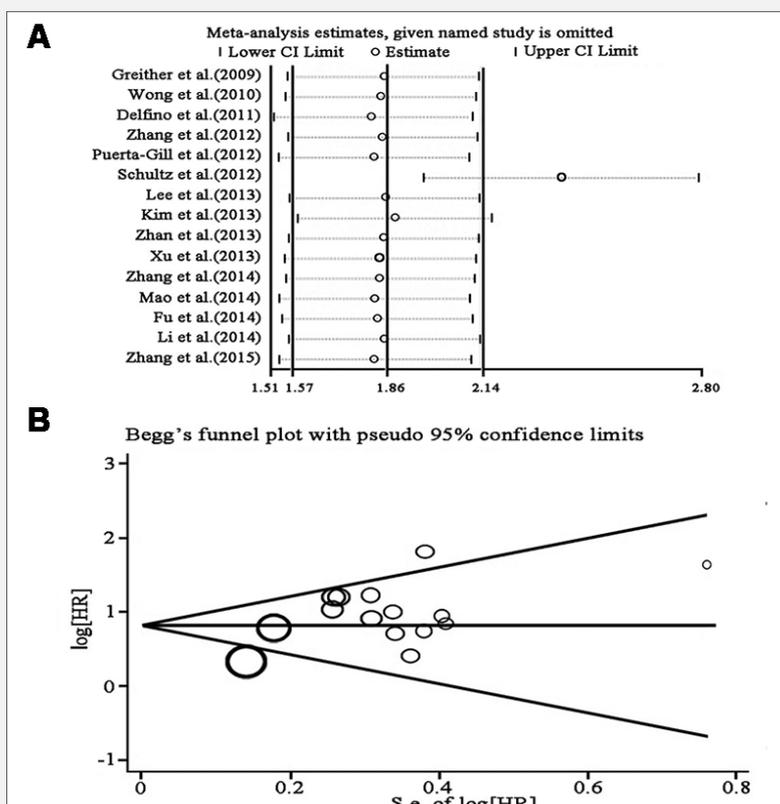


Figure 4. (A) Sensitivity analysis for meta-analysis of miR-222. (B) Funnel plot of higher miR-222 expression and overall survival in cancer patients. Circles indicate included studies.

fects model) (Figure 3). Subgroup analysis was performed to evaluate the effect of miR-222 due to the heterogeneity. In the subgroup of Asian people and digestive system malignancies, upregulated miR-222 expression was associated with poor DFS (HR = 2.67, 95% CI, 1.60 - 3.74;  $p < 0.001$ ) without heterogeneity ( $I^2 = 14\%$ ,  $P_{\text{heterogeneity}} = 0.281$ ).

### Sensitivity analysis

Sensitivity analysis was carried out by sequentially omitting individual studies using a fixed model (Figure 4A). The study conducted by Schultz et al. was detected to be an outlier to the analysis and influenced the overall results. When this study was excluded from the analysis, a similar result was observed (HR = 2.39, 95% CI, 1.91 - 2.80;  $p < 0.001$ ) without heterogeneity ( $I^2 = 0\%$ ,  $P_{\text{heterogeneity}} = 0.678$ ), which illustrated the reliability of the analysis.

### Publication bias

Begg's funnel plot was applied to evaluate publication bias (Figure 4B). The p-values of Begg's for OS was over 0.05 ( $p = 0.81$  for Begg's test). No publication bias was revealed.

## DISCUSSION

In the past few years, miRNAs have been considered as potential biomarkers for cancer diagnosis and prognosis for their distinct expression in cancer tissues and easy detection. Recently, a number of studies revealed the aberrant expression of miR-222 in multiple malignant tumors, such as glioma, glioblastoma, breast, hepatocellular, gastric, bladder, prostate, pancreatic, and thyroid cancer [9-13,21-23,32-33]. It acts as an onco-miR in the development of various malignancies by targeting certain genes, and its high expression is associated with a relatively poor prognosis (Table 2). In breast cancer, miR-222 promotes aggressiveness of cancer cells by accelerating G1/S transition of the cell cycle [9]. Furthermore, upregulation of miR-222 induces epithelial to mesenchymal transition in breast cancer cells by suppressing adiponectin receptor 1 (AdipoR1), which indicates that overexpression of miR-222 occurs in poorly-differentiated and more aggressive tumor phenotypes [34]. In gastric cancer, miR-222 promotes cell proliferation and colony formation by inhibiting reversion-inducing cysteine-rich protein with kazal motifs (RECK) directly [36]. MiR-222 is confirmed to inhibit cell apoptosis by targeting the pro-apoptotic molecule p53 up-regulated modulator of apoptosis (PUMA) in glioblastoma [32]. It also increases invasiveness of glioblastoma cells through downregulating protein tyrosine phosphatase  $\mu$  (PTP $\mu$ ) [39]. Similarly, increased miR-222 is able to reduce p27Kip1 levels in thyroid carcinoma, which induces cells to progress to the S phase of the cell cycle [33]. These findings may partly explain the link between high miR-222 expression and poor outcomes. In

addition, it has been reported that miR-222 confers radioresistance on glioblastoma cells through activating Akt [44], which makes miR-222 a possible target for regulating the radiosensitivity of malignancies. However, there are some inconsistent views that cannot be ignored. MiR-222 is downregulated in prostate cancer samples compared with the benign prostatic hyperplasia samples by performing miRNA array hybridization [45]. Similarly, miR-222 is decreased in prostate cancer, and ectopic expression of it reduces cancer cell growth [46]. Another study verifies the decreased expression of miR-222 in bladder cancer tumor tissues [47]. In gastrointestinal stromal tumors, miR-222 is reduced compared to control tissue, and its overexpression induces apoptosis by a signaling cascade involving KIT, Akt, and BCL2 [48]. Hence, the roles of miR-222 are complicated and much remains to be resolved to completely understand the exact mechanisms of miR-222 in cancer development and its application in clinical practice.

In the first meta-analysis of miR-222 related to the outcomes of various cancers, Wang et al. retrieved 4 studies and found that high miR-222 expression is negatively correlated with the clinical outcome, with a pooled HR of 2.15 [49]. Nevertheless, the relatively small study size limited the power of the results. Here, we have updated the meta-analysis using 16 studies involving 1,354 patients to further validate the relationship between miR-222 and patient outcomes. We found that elevated expression of miR-222 was associated with poor OS, with a pooled HR of 1.86 (95% CI, 1.57 - 2.14), which was consistent with the previous study and further confirmed that miR-222 could act as a predictive marker for prognosis. Since no heterogeneity was revealed, we did not carry out subgroup analysis.

The prognostic value of miR-222 in DFS was also examined in 3 studies involving 266 patients. Evidence for a relationship between miR-222 expression and patient survival was insufficient, with a pooled HR of 1.97 (95% CI, 0.40 - 3.53). By performing subgroup analysis, the study by Schaefer et al. apparently affected the overall outcomes, which was the main source of heterogeneity. As the only study focused on prostate carcinoma, Schaefer et al. revealed that miR-222 was downregulated in cancer tissues, which might reflect the anti-cancer aspect of miR-222. This may partly explain the reduced hazard risk of high miR-222 expression in prostate cancer. Due to the limited number of studies we cannot conclusively deny the relationship, and more large-scale investigations are needed.

There are some limitations in our study that should be pointed out. First, we did not investigate the relationship between miR-222 and biological subtypes of a certain cancer for the reason that such distinction was unavailable in the majority of studies. Second, the cutoff point to define high and low miR-222 expression differed among the included studies, which might affect the effectiveness of miR-222 in malignancy prognosis as a predictive factor. Thirdly, a significant heterogeneity was detected in the analysis for DFS, so we should be

cautious when interpreting the results and the following subgroup analysis in Asian people and digestive system malignancies due to the relatively small sample size of the research. And last, we did not evaluate the relationship between miR-222 deregulation and MFS since only one study mentioned it.

## CONCLUSION

Our meta-analysis demonstrated that high expression of miR-222 predicts relatively poor survival in various cancers, especially OS. This result offers new insight into exploiting miR-222 as a potential biomarker for cancer prognosis. More large-scale data are needed to better understand the exact role of miR-222, especially circulating miR-222 level, and to make the best use of it in clinical application.

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### Declaration of Interest:

The authors have no relevant affiliations or financial involvement with any organization or entity with a financial conflict with any materials discussed in the paper. All authors declare that there is no conflict of interest regarding the publication of this work.

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