IVYSPRING INTERNATIONAL PUBLISHER

Journal of Cancer

2019; 10(8): 1825-1832. doi: 10.7150/jca.29438

Research Paper

Dysregulated Expression of Circular RNAs Serve as Prognostic and Clinicopathological Markers in Cancer

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Received: 2018.08.23; Accepted: 2019.02.07; Published: 2019.04.21

Abstract

Purpose: Circular RNAs (circRNAs) as prognostic biomarkers have spurred considerable interest in several types of tumors. In the present study, we aimed to elucidate the clinicopathological and prognostic values of circRNAs in human cancer.

Methods: We systematically searched PubMed Central (PMC), PubMed, Web of Science, EMBASE, Scopus, CBM and the Cochrane Library databases up to Nov 29, 2018. Eligible studies reporting on the association between circRNAs expression and clinicopathological and prognostic outcomes in cancer were incorporated. Pooled odds ratios (ORs) and 95% confidence intervals (CIs) were used to assess clinicopathological parameters, and hazard ratios (HRs) and 95% CIs to estimate overall survival (OS).

Results: Thirty-two studies involving 4529 patients were incorporated into our meta-analysis. Pooled results showed that high expression of oncogenic circRNAs was significantly associated with poor clinicopathological characteristics (tumor size: OR=1.29, 95%CI: 1.10-1.51; TNM stage: OR=1.62, 95%CI: 1.41-1.87; differentiation grade: OR=1.41, 95%CI: 1.11-1.78; lymph node metastasis: OR=1.69; 95%CI: 1.34-2.13; distant metastasis: OR=2.75; 95%CI: 1.92-3.95) and a poor prognosis (OS: HR=2.75; 95%CI: 2.34-3.15). Furthermore, we found that high expression of tumor-suppressor circRNAs was correlated with improved clinical characteristics (tumor size: OR=0.72; 95%CI: 0.56-0.92; TNM stage: OR=0.77, 95%CI: 0.68-0.88) and longer survival times (OS: HR=0.49; 95%CI: 0.42-0.56). Subgroup analyses based on cancer types and circRNA types were also performed.

Conclusion: Our study indicates that circRNAs may serve as important biomarkers for clinicopathologic features and prognosis in human cancer.

Key words: circRNA, cancer, prognosis, meta-analysis

Introduction

Circular RNA (circRNA) is a new class of endogenous non-coding RNA generated from the back-splicing by the canonical spliceosome [1]. Numerous circRNAs seem to be specifically expressed in a given cell type or developmental stage [2]. CircRNAs are characterized by a covalently closed loop structure with neither a 5′ cap nor a 3′ polyadenylated tail [3, 4]. Moreover, they are inherently resistant to exonucleolytic RNA decay. Taken their conserved and stable characteristics into account, circRNAs might be suitable as required novel biomarkers and therapeutic targets for human cancer [5-7]. Recent studies indicate that circRNAs might regulate transcription process and RNA splicing, function as efficient microRNA sponges, and can be translated into protein driven by N6-methyladenosine (m6A) modification [8, 9]. However, more underlying mechanisms and functions of circRNAs remain largely unknown. CircRNAs have been recently confirmed to have regulative functions in cell function, development of heart diseases, and pathogenesis of neurodegenerative diseases such as

Alzheimer's disease [10]. Cancer is a major public health problem worldwide [11, 12]. The function of upregulated or downregulated circRNAs in various cancer types still require further investigation.

In this study, we performed a meta-analysis to summarize the clinicopathological and prognostic values of circRNAs in different types of cancer. Further prospective studies including more kinds of circRNAs in various tumors are warranted in the future.

Methods

Data search strategy

A computerized literature search was performed in the PubMed Central (PMC), PubMed, Web of Science, EMBASE, Scopus, CBM and the Cochrane Library databases up to Nov 29, 2018. A search strategy was developed based on the following terms: ("circRNA" or "circular RNA") and ("cancer" or "carcinoma" or "tumor" or "tumour" or "neoplas*"). We additionally hand-searched the references of relevant articles and contacted investigators of certain studies when necessary. To be eligible for inclusion in the meta-analysis, a study must meet the following criteria: (1) case–control study or cohort study; (2) patients had a pathological diagnosis of cancer; (3) assessing the association between circRNA expression, clinicopathological features, and prognosis. Exclusion criteria were as follows: (1) literatures not pertinent to circRNA or cancer; or (2) similar studies from the same author as well as multiple duplicate data in the different works; or (3) animal experiments, case reports, correspondences, reviews, expert opinions, letters; or (4) no available data and the authors could not be contacted.

Data extraction and quality assessment

Two investigators (XH, ZCZ) evaluated the eligibility of all retrieved studies and extracted the relevant data independently. Extracted databases were then cross-checked between the two authors to rule out any discrepancy. Disagreement was resolved by consulting with a third investigator (ZWS). The following data of each collected studies were extracted independently: author, year of publication, circRNA type, cancer type, cases, detection method, role of circRNA and duration of follow-up. The study quality was assessed in accordance with the Newcastle-Ottawa Scale (NOS) (Supplementary Table S1). Eight items were extracted, and each item scored 1. The total scores ranged from 0 to 8. If the scores were≥7, then the study was considered high quality. Our investigation process was in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement.

Statistical analysis

The statistical analysis was performed using STATA 14. Pooled odds ratios (ORs) and 95% confidence intervals (CIs) were used to assess clinicopathological parameters, and hazard ratios (HRs) and 95% CIs to estimate overall survival (OS). The between-study heterogeneity was evaluated by using the chi-square test and the I^2 statistic. An I^2 value of >50% of the I^2 statistic was considered to indicate significant heterogeneity [13]. When a significant heterogeneity existed across the included studies, a random effects model was used for the analysis. Otherwise, the fixed effects model was used [14]. Subgroup analyses were performed to detect the source of heterogeneity. We further conducted sensitivity analyses to substantiate the stability of results and detect the potential source of heterogeneity. Publication bias was evaluated qualitatively by inspecting funnel plots and quantitatively through the Begg's and Egger's test. A two-tailed P-value<0.05 implies a statistically significant publication bias.

Results

Search results

The study selection process is illustrated in Fig. 1. A total of 248 potential articles were identified from the databases search. Among these articles, 180 were excluded after abstract review, leaving 68 articles for the full-text review. In the review, 36 studies were excluded for the reasons as follows: eleven were eliminated because they were irrelevant to circRNA or cancer, twelve studies were of no relevant outcomes reported, six studies were of reviews, four studies involved non-human experiments, and three studies were excluded because of insufficient data for analysis. Finally, thirty-two studies with a total of 4529 patients that met the inclusion criteria were included in this meta-analysis.

Study selection and characteristics

Baseline characteristics of the included studies are presented in Table 1. The publication years of the eligible studies ranged from 2017 to 2018. Cancer types included gastric cancer (n=2), colorectal cancer (n=3), hepatocellular carcinoma (n=6), breast cancer (n=2), bladder cancer (n=5), lung cancer (n=4), osteosarcoma (n=5). The number of patients in each study ranged from 30 to 631. Additionally, the circRNA expression levels were measured by quantitative real time polymerase chain reaction (qRT-PCR). As indicated in Table 1, twenty-one circRNAs were recognized as tumor promoters and eleven were tumor suppressors. Moreover, the mean

duration of follow-up ranged from 33 to 140 months. CircRNAs could serve as sponges to regulate gene expression via sequestering miRNAs. Therefore, we included corresponding miRNAs. All included studies screened out circRNAs from tumor tissues. According to the Newcastle-Ottawa Scale (NOS), the quality scores of the included trials ranged from 7 to 8, which indicated a high quality (Additional file 1).

Meta-analysis for clinicopathological features

In the present study, we assessed the relationship between circRNAs expression and clinicopathological features of cancer patients (Table 2). High expression of oncogenic circRNAs was significantly associated with poor clinicopathological characteristics (tumor size: OR=1.29, 95%CI: 1.10-1.51; TNM stage: OR=1.62, 95%CI: 1.41-1.87; differentiation grade: OR=1.41, 95%CI: 1.11-1.78; lymph node metastasis: OR=1.69; 95%CI: 1.34-2.13; distant metastasis: OR=2.75; 95%CI: 1.92-3.95). Furthermore, our study showed that high expression of tumor-suppressor circRNAs was correlated with improved clinical characteristics (tumor size: OR=0.72; 95%CI: 0.56-0.92; TNM stage: OR=0.77, 95%CI: 0.68-0.88). However, no significant relationship was observed between tumor-suppressor circRNAs overexpression and other clinical characteristics such as age, gender, differentiation grade, lymph node metastasis and distant metastasis.

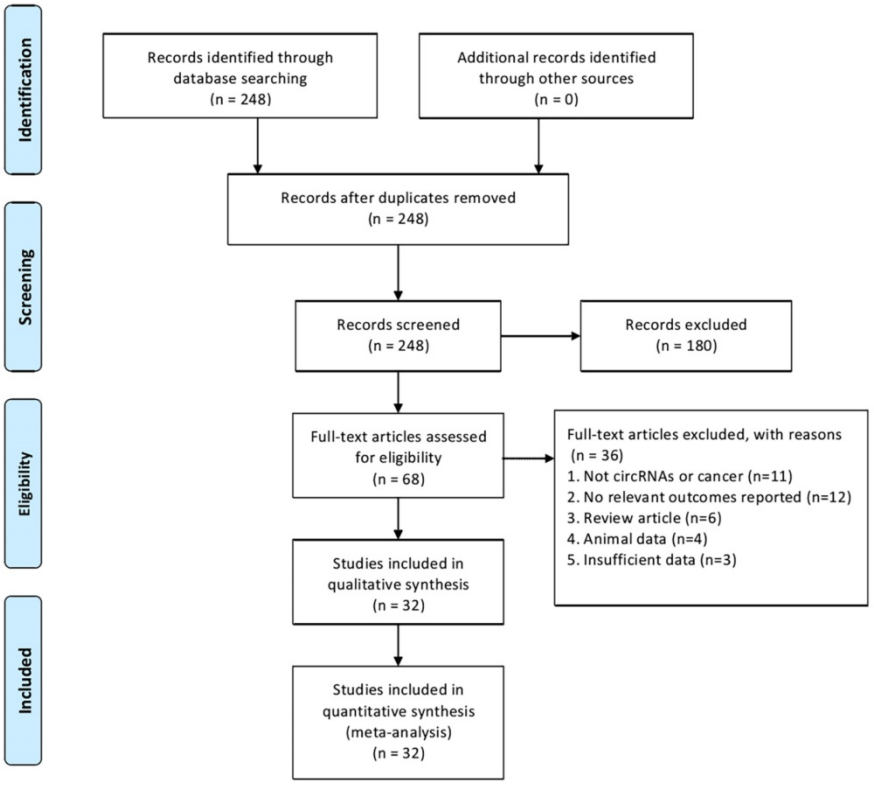

Figure 1. Flowchart of the study selection process.

Meta-analysis for overall survival

As depicted in Fig. 2, high expression of oncogenic circRNAs was significantly associated with a poor prognosis (OS: HR=2.75; 95%CI: 2.34-3.15; p<0.001), and the fixed-effect model was adopted in terms of no significant heterogeneity among the studies (I^2=0.5%, p=0.452). Furthermore, high expression of tumor-suppressor circRNAs was correlated with longer survival times (OS: HR=0.49; 95%CI: 0.42-0.56; p<0.001). No significant heterogeneity among the studies (I^2=43.5%, p=0.061) was found and the fixed-effect model was adopted (Fig. 3).

Subgroup analysis in terms of various cancer types

We further conducted subgroup analysis by factors of cancer types to explore the source of heterogeneity (Table 3). High expression of circRNAs was correlated with longer survival times in gastric cancer (OS: HR=0.62; 95%CI: 0.50-0.74), hepatocellular carcinoma (OS: HR=0.44; 95%CI: 0.33-0.55), bladder cancer (OS: HR=0.49; 95%CI: 0.33-0.65) and osteosarcoma (OS: HR=0.49; 95%CI: 0.28-0.71). However, high expression of circRNAs was correlated with poor survival in colorectal cancer (OS: HR=2.52; 95%CI: 1.61-3.43), breast cancer (OS: HR=3.47; 95%CI: 1.95-5.00) and lung cancer (OS: HR=2.91; 95%CI: 1.92-3.91). Relatively significant heterogeneities were observed in hepatocellular carcinoma (I^2= 86.9%), lung cancer (I^2= 71.0%) and osteosarcoma (I^2= 70.3%).

Subgroup analysis in terms of various circRNAs types

When subgrouped by circRNAs types (Table 4), our study found that high expression of circRNAs was correlated with longer survival times in circPVT1 (OS: HR=0.54; 95%CI: 0.35-0.74), circHIPK3 (OS: HR=0.50; 95%CI: 0.29-0.72), circ_0001649 (OS: HR= 0.35; 95%CI: 0.20-0.51) and circITCH (OS: HR=0.49; 95%CI: 0.30-0.69). However, high expression of circRNAs was correlated with poor survival in circRNA Cdr1as (OS: HR=2.77; 95%CI: 1.70-3.83) and circ_0067934 (OS: HR=3.66; 95%CI: 2.15-5.16). No significant heterogeneities were observed in circRNA Cdr1as (I^2=46.3%), circITCH (I^2=0.0%) and circ_0067934 (I^2=0.0%).

Table 1. Main characteristics of the studies included in this meta-analysis.

Study	Year	CircRNA	Cancer type	mRNA	Sample	CircRNA expression		Detection method	Expression status	Follow -up (months)	Cita-tion
						High	Low				
Zhou et al.	2018	circ_0008717	Osteosarcoma	miR-203	Tumor tissue	45	45	qRT-PCR	Up-regulated	80	[15]
Zhu et al.	2018	circPVT1	Osteosarcoma	NA	Tumor tissue	30	50	qRT-PCR	Up-regulated	62	[16]
Zhang et al.	2017	circUBAP2	Osteosarcoma	miR-143	Tumor tissue	42	50	qRT-PCR	Up-regulated	60	[17]
Hsiao et al.	2017	circCCDC66	Colorectal cancer	miR-33b, miR-93	Tumor tissue	131	98	qRT-PCR	Up-regulated	58	[18]
Weng et al.	2018	ciRS-7	Colorectal cancer	miR-7	Tumor tissue	89	76	qRT-PCR	Up-regulated	83	[19]
He et al.	2017	circGFRA1	Breast cancer	miR-34a	Tumor tissue	109	103	qRT-PCR	Up-regulated	140	[20]
Jiang et al.	2017	circCdr1as	Cholangiocarcinoma	NA	Tumor tissue	24	30	qRT-PCR	Up-regulated	45	[21]
Zhong et al.	2017	circMYLK	Bladder cancer	miR-29a	Tumor tissue	16	16	qRT-PCR	Up-regulated	33	[22]
Liu et al.	2018	circ_103809	Lung cancer	miR-4302	Tumor tissue	22	22	qRT-PCR	Up-regulated	76	[23]
Yao et al.	2017	circ_100876	Lung cancer	NA	Tumor tissue	48	52	qRT-PCR	Up-regulated	40	[24]
Zhao et al.	2017	circFADS2	Lung cancer	miR-498	Tumor tissue	20	23	qRT-PCR	Up-regulated	60	[25]
Luan et al.	2018	circ_0084043	Melanoma	miR-153-3p	Tumor tissue	15	15	qRT-PCR	Up-regulated	60	[26]
Wei et al.	2018	circZFR	Papillary thyroid cancer	miR-1261	Tumor tissue	41	41	qRT-PCR	Up-regulated	55	[27]
Zhang et al.	2018	circ_0023404	Cervical cancer	miR-136	Tumor tissue	27	26	qRT-PCR	Up-regulated	78	[28]
Verduci et al.	2017	circPVT1	Head and neck squamous cell carcinoma	miR-497-5p	Tumor tissue	71	35	qRT-PCR	Up-regulated	70	[29]
Xu et al.	2017	circCdr1as	Hepatocellular carcinoma	miR-7	Tumor tissue	48	47	qRT-PCR	Up-regulated	62	[30]
Zeng et al.	2017	circHIPK3	Colorectal cancer	miR-7	Tumor tissue	89	89	qRT-PCR	Up-regulated	90	[31]
Li et al.	2017	circHIPK3	Bladder cancer	miR-558	Tumor tissue	45	179	qRT-PCR	Up-regulated	112	[32]
Meng et al.	2018	circ_10720	Hepatocellular carcinoma	NA	Tumor tissue	32	65	qRT-PCR	Up-regulated	118	[33]
Wu et al.	2018	circIRAK3	Breast cancer	miR-3607	Tumor tissue	60	62	qRT-PCR	Up-regulated	120	[34]
Wang et al.	2018	circ_0067934	Lung cancer	NA	Tumor tissue	79	80	qRT-PCR	Up-regulated	60	[35]
Zhu et al.	2018	circ_0067934	Hepatocellular carcinoma	miR-1324	Tumor tissue	25	25	qRT-PCR	Up-regulated	60	[36]
Chen et al.	2017	circPVT1	Gastric cancer	miR-125	Tumor tissue	107	80	qRT-PCR	Down-regulated	85	[37]
Zhang et al.	2017	circLARP4	Gastric cancer	miR-424-5p	Tumor tissue	220	411	qRT-PCR	Down-regulated	110	[38]
Han et al.	2017	circMTO1	Hepatocellular carcinoma	miR-9	Tumor tissue	116	116	qRT-PCR	Down-regulated	80	[39]
Zhang et al.	2018	circ_0001649	Hepatocellular carcinoma	NA	Tumor tissue	35	42	qRT-PCR	Down-regulated	44	[40]
Yang et al.	2018	circITCH	Bladder cancer	miR-17, miR-224	Tumor tissue	25	45	qRT-PCR	Down-regulated	60	[41]
Wu et al.	2018	circ_0002052	Osteosarcoma	miR-1205	Tumor tissue	54	54	qRT-PCR	Down-regulated	50	[42]
Ma et al.	2018	circHIPK3	Osteosarcoma	NA	Tumor tissue	37	45	qRT-PCR	Down-regulated	60	[43]
Okholm et al.	2017	circHIPK3	Bladder cancer	NA	Tumor tissue	228	229	qRT-PCR	Down-regulated	75	[44]
Okholm et al.	2017	circCDYL	Bladder cancer	NA	Tumor tissue	228	229	qRT-PCR	Down-regulated	75	[44]
Xing et al.	2018	circ_0001649	Retinoblastoma	NA	Tumor tissue	30	30	qRT-PCR	Down-regulated	60	[45]
Guo et al.	2017	circITCH	Hepatocellular carcinoma	NA	Tumor tissue	100	188	qRT-PCR	Down-regulated	83	[46]

Abbreviations: qRT-PCR, quantitative real time polymerase chain reaction; NA, not available.

Table 2. Clinical characteristics of circRNAs in cancer.

	Tumor promoter			Tumor suppressor		
	OR	95% CI	P	OR	95% CI	P
Age	0.794	0.592-1.065	0.124	1.008	0.804-1.263	0.946
Gender (M/W)	1.264	0.879-1.817	0.207	1.020	0.896-1.161	0.763
Tumor size	**1.291**	**1.104-1.510**	**0.001**	**0.717**	**0.560-0.917**	**0.008**
TNM stage (III+IV/I+II)	**1.621**	**1.407-1.868**	**0.000**	**0.773**	**0.683-0.875**	**0.000**
Differentiation grade	**1.406**	**1.112-1.778**	**0.004**	0.889	0.760-1.040	0.141
Lymph node metastasis (Y/N)	**1.687**	**1.337-2.129**	**0.000**	0.993	0.889-1.110	0.906
Distant metastasis (Y/N)	**2.753**	**1.919-3.949**	**0.000**	0.608	0.360-1.027	0.063

Abbreviations: M, men; W, women; Y, yes; N, no; OR, odds ratio; CI, confidence interval. The results are in bold if P < 0.05.

Table 3. Subgroup analysis of circRNAs in various cancer types.

Subgroup analysis	Studies (n)	CircRNA	HR	95% CI	*p*-value	Heterogeneity		
						I^2 (%)	P_Q	Model
Gastric cancer	Chen et al. (2017)	circPVT1	0.508	0.347-0.745				
	Zhang et al. (2017)	circLARP4	0.689	0.552-0.860				
	Total		**0.621**	**0.500-0.743**	**0.000**	**49.6%**	**0.159**	**Fixed**
Colorectal cancer	Hsiao et al. (2017)	circCCDC66	2.266	1.265-4.061				
	Weng et al. (2018)	ciRS-7	2.441	1.298-4.594				
	Zeng et al. (2017)	circHIPK3	3.047	1.525-5.147				
	Total		**2.518**	**1.608-3.429**	**0.000**	**0.0%**	**0.809**	**Fixed**
Hepatocellular carcinoma	Han et al. (2017)	circMTO1	0.491	0.349-0.691				
	Zhang et al. (2018)	circ_0001649	0.265	0.141-0.498				
	Meng et al. (2018)	circ_10720	4.300	1.495-6.984				
	Xu et al. (2017)	circCdr1as	3.621	2.108-5.325				
	Guo et al. (2017)	circITCH	0.512	0.320-0.781				

Subgroup analysis	Studies (n)	CircRNA	HR	95% CI	*p*-value	Heterogeneity		
						I^2 (%)	P_Q	Model
	Zhu et al. (2018)	circ_0067934	3.605	1.816-5.546				
	Total		**0.441**	**0.333-0.549**	**0.000**	**86.9%**	**0.000**	**Random**
Breast cancer	He et al. (2017)	circGFRA1	3.790	2.011-7.142				
	Wu et al. (2018)	circIRAK3	3.328	1.208-5.234				
	Total		**3.474**	**1.947-5.000**	**0.000**	**0.0%**	**0.764**	**Fixed**
Bladder cancer	Yang et al. (2018)	circITCH	0.480	0.236-0.976				
	Zhong et al. (2017)	circMYLK	2.595	1.010-6.668				
	Okholm et al. (2017)	circHIPK3	0.406	0.220-0.750				
	Okholm et al. (2017)	circCDYL	0.533	0.325-0.780				
	Li et al. (2017)	circHIPK3	4.325	2.800-6.907				
	Total		**0.490**	**0.332-0.654**	**0.000**	**71.0%**	**0.008**	**Random**
Lung cancer	Liu et al. (2018)	circ_103809	2.494	1.036-6.005				
	Yao et al. (2017)	circ_100876	2.731	1.709-4.363				
	Zhao et al. (2017)	circFADS2	3.232	1.495-6.984				
	Wang et al. (2018)	circ_0067934	3.774	1.498-6.670				
	Total		**2.913**	**1.919-3.907**	**0.000**	**0.0%**	**0.883**	**Fixed**
Osteosarcoma	Wu et al. (2018)	circ_0002052	0.406	0.220-0.750				
	Ma et al. (2018)	circHIPK3	0.461	0.218-0.977				
	Zhou et al. (2018)	circ-0008717	2.729	1.100-6.773				
	Zhu et al. (2018)	circPVT1	3.306	1.663-6.570				
	Zhang et al. (2017)	circUBAP2	2.364	1.275-4.382				
	Total		**0.496**	**0.282-0.710**	**0.000**	**70.3%**	**0.009**	**Random**

Abbreviations: HR, hazard ratio; CI, confidence interval.

Table 4. Subgroup analysis in terms of various circRNAs types.

Subgroup analysis	Studies (n)	Cancer type	HR	95% CI	*p*-value	Heterogeneity		
						I^2 (%)	P_Q	Model
circPVT1	Chen et al. (2017)	Gastric cancer	0.508	0.347-0.745				
	Zhu et al. (2018)	Osteosarcoma	3.306	1.663-6.570				
	Verduci et al. (2017)	Head and neck squamous cell carcinoma	2.120	1.213-4.950				
	Total		**0.544**	**0.347-0.741**	**0.000**	**73.8**	**0.022**	**Random**
circHIPK3	Zeng et al. (2017)	Colorectal cancer	3.012	1.534-5.052				
	Okholm et al. (2017)	Bladder cancer	0.406	0.220-0.750				
	Li et al. (2017)	Bladder cancer	4.011	2.856-6.901				
	Ma et al. (2018)	Osteosarcoma	0.461	0.218-0.977				
	Total		**0.502**	**0.287-0.716**	**0.000**	**84.7**	**0.000**	**Random**
circRNA Cdr1as	Xu et al. (2017)	Hepatocellular carcinoma	3.612	2.109-5.315				
	Jiang et al. (2017)	Cholangiocarcinoma	2.108	1.120-3.968				
	Total		**2.767**	**1.704-3.831**	**0.000**	**46.3**	**0.172**	**Fixed**
circ_0001649	Zhang et al. (2018)	Hepatocellular carcinoma	0.265	0.141-0.498				
	Xing et al. (2018)	Retinoblastoma	0.611	0.335-0.901				
	Total		**0.353**	**0.199-0.506**	**0.000**	**71.8**	**0.060**	**Random**
circ-ITCH	Guo et al. (2017)	Hepatocellular carcinoma	0.500	0.320-0.780				
	Yang et al. (2018)	Bladder cancer	0.480	0.236-0.976				
	Total		**0.494**	**0.299-0.690**	**0.000**	**0.0**	**0.927**	**Fixed**
circ_0067934	Zhu et al. (2018)	Hepatocellular carcinoma	3.635	1.821-5.508				
	Wang et al. (2018)	Lung cancer	3.774	1.498-6.670				
	Total		**3.659**	**2.154-5.164**	**0.000**	**0.0**	**0.915**	**Fixed**

Abbreviations: HR, hazard ratio; CI, confidence interval.

Publication bias and sensitivity analysis

The funnel plot did not indicate any evidence of publication bias in this analysis (Figure S2). No evidence of publication bias was observed from Begg's funnel plot (P=0.369) (Figure S3) and Egger's test (P=0.082) (Figure S4). To sum up, the possibility of publication bias could be excluded. The sensitivity analysis showed that the results of the meta-analysis did not change when studies were omitted one by one (Figure S5).

Discussion

The present study revealed a significant association between high expression of circRNAs and clinicopathological and prognostic significance in human cancer. Thirty-two studies involving 4529 patients were incorporated into our meta-analysis. Since the expression of circRNAs were upregulated or downregulated in different cancers, we decided to recognize twenty-one circRNAs as tumor promoters and eleven as tumor suppressors and analysis them respectively. Pooled results showed that high expression of oncogenic circRNAs was significantly associated with poor clinicopathological characteristics including tumor size, TNM stage, differentiation grade, lymph node metastasis and distant metastasis. A significant association between oncogenic circRNAs

and a poor prognosis was also detected in our study. Furthermore, we found that high expression of tumor-suppressor circRNAs was correlated with longer survival times and improved clinical characteristics such as tumor size and TNM stage.

Relatively significant heterogeneities were observed in our study. To explore the source of heterogeneity, we performed sensitivity analysis and found that none of those studies altered the pooled OR significantly, indicating that other unknown factors might be the cause. Furthermore, we predicted that disease type may account for the heterogeneity and the stratified analyses were then performed. Subgroup analysis focused mainly on seven cancer types, including gastric cancer [37, 38], colorectal cancer [18, 19, 31], hepatocellular carcinoma [30, 33, 36, 39, 40, 46], bladder cancer [22, 32, 41, 44], breast cancer [20, 34], lung cancer [23-25, 35], osteosarcoma [15-17, 42, 43]. Because of only one article included for other cancer types, we failed to perform further meta-analysis. Relatively significant heterogeneities were observed in three cancer types including hepatocellular carcinoma, lung cancer and osteosarcoma (I^2>50%). Small sample size and limited article included may account for the significant heterogeneity. Neither the Egger test nor the Begg's funnel plot showed significant publication bias for the association between circRNAs expression and clinicopathological and prognostic significances. Even though the results are reliable, additional relevant studies are warranted to further confirm the findings of this meta-analysis.

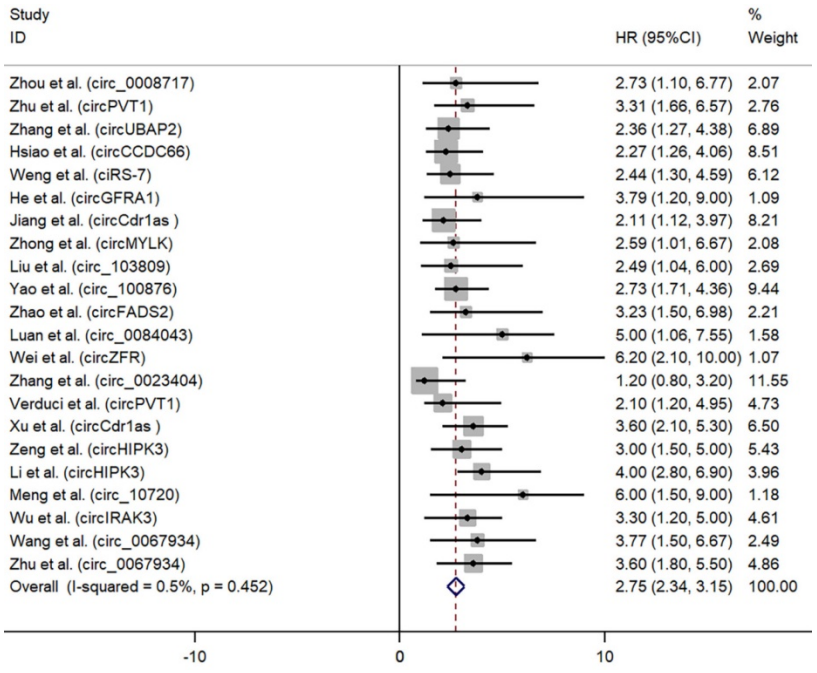

Figure 2. Forest plots for OS according to the type of oncogenic circRNAs in cancer.

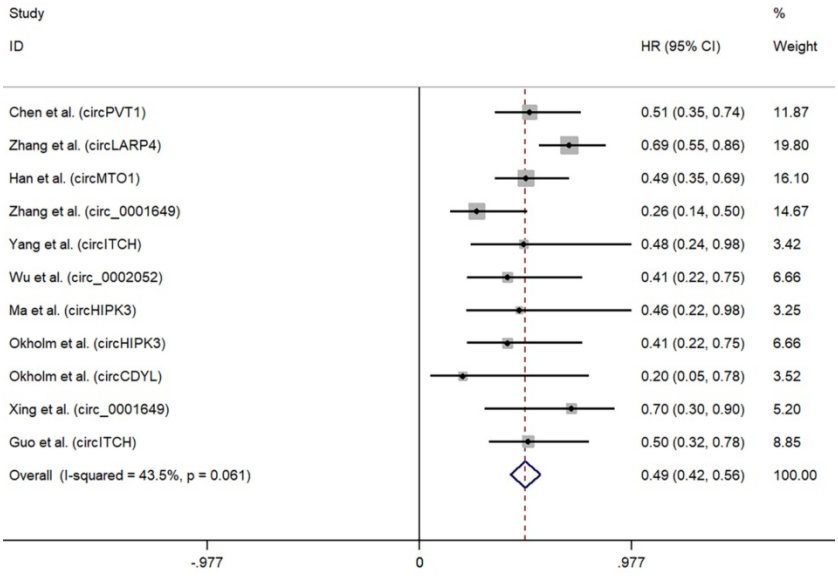

Figure 3. Forest plots for OS according to the type of tumor suppressor circRNAs in cancer.

Four previous meta-analysis by Wang *et al.* [5], Ding *et al.* [47], Li *et al.* [48], and Chen *et al.* [49] were also performed to detect the association between circRNAs and cancer. As for Li *et al.*, they included 10 articles about circRNAs as diagnostic biomarkers for cancer. In the study of Wang *et al.*, they highlighted the diagnostic value of circRNAs for human cancers especially in HCC diagnosis with 17 publications. Chen *et al.* just focused on circRNAs as potential biomarkers for the diagnosis of digestive system malignancy. Li *et al.*, Wang *et al.*, and Chen *et al.* failed to discuss anything about the prognostic and clinicopathological significances of circRNAs. Moreover, limited studies and sample sizes were included in their studies, which decreased the reliability of conclusions. Ding *et al.* assessed the expression of circRNAs as a promising biomarker in the diagnosis and prognosis of cancers. However, only 11 articles were included in the prognostic meta-analysis. In our study, a computerized literature search was performed and thirty-two studies involving 4529 patients were included. Moreover, we assessed both prognostic and clinicopathological significance

of circRNAs expression in cancer patients. A further subgroup analysis in different cancer types were also performed. Nevertheless, large-scale and better-designed trials are warranted to further identify the clinicopathological and prognostic significance of circRNAs expression in cancer.

Limitations

Despite the promising data, some limitations still should be acknowledged. Firstly, because of limited number of studies, we failed to perform subgroup analysis in terms of different kinds of circRNAs. More circRNAs types and other aspects of cancer including chemotherapeutic susceptibility and relapse should be explored. Secondly, functional studies are needed to clarify the underlying mechanisms of circRNAs in the tumorigenesis. Thirdly, the extensive clinical application of circRNA requires further study. Moreover, the number of subjects in the included studies are relatively small, which might result in a lack of statistical power and prevent a meaningful analysis of the results. With the updating of gene chip and microarray platform technology and an explosion of circRNAs research in cancer, a significant extension of our finding and re-analysis including more patients, could be accomplished in near future. Finally, when not reported in original articles, HRs were extrapolated from the Kaplan-Meier curves or calculated from the provided data within the papers according to the method of Parmar *et al.* [50], which could introduce potential source of bias. However, this practice has not been shown to yield results significantly different from direct methods of HR estimation.

Conclusions

The present meta-analysis suggests a significant association between high expression of circRNAs and clinicopathological and prognostic significance in human cancer. Additionally, circRNAs may be promising biomarkers and therapeutic targets for cancer. Nevertheless, large-scale studies using standardized approaches are warranted to provide a new insight into the prognostic value of circRNAs.

Supplementary Material

Supplementary figures and tables.
http://www.jcancer.org/v10p1825s1.pdf

Abbreviations

CircRNA, Circular RNA; OR, Odds ratio; CI, Confidence interval; HR, Hazard ratios; OS, Overall survival; NOS, Newcastle-Ottawa Scale; PRISMA, Preferred Reporting Items for Systematic Reviews and Meta-Analyses; qRT-PCR, quantitative real time polymerase chain reaction.

Acknowledgements

This work is supported by the National Key Research and Development Program of China (2016YFC1100100), the Major Research Plan of National Natural Science Foundation of China (No. 91649204), and the Natural Science Foundation of Hubei Province, China (No. 2018CFB118).

Competing Interests

The authors have declared that no competing interest exists.

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