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ORIGINAL ARTICLE

The Impact of MicroRNA-133a on Prognosis and Clinicopathological Parameters for Digestive System Cancers: a Comprehensive Study Based on Meta-Analysis and TCGA Database

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Abstract

We conducted a meta-analysis on the impact of microRNA-133a (miR-133a) on digestive system cancers, and verified the results through The Cancer Genome Atlas (TCGA). Relevant studies were searched in English and Chinese database and meta-analysis was performed using Stata 12.0. The corresponding information of miR-133a and digestive system cancers were obtained from TCGA database and analysis was performed using SPSS. Increased miR-133a expression was linked with favorable overall survival (OS) in digestive system cancers (HR = 0.539, 95% CI: 0.416–0.698, $P < 0.001$), digestive tract cancers (HR =0.558, 95% CI: 0.406–0.767, $P < 0.001$), esophageal squamous cell carcinoma (ESCC) (HR = 0.427, 95% CI: 0.265–0.690, $P = 0.001$) and gastric cancer (HR = 0.541, 95% CI: 0.385–0.761, $P < 0.001$). The expression of miR-133a was significantly lower in cancer tissue compared with adjacent tissue for ESCC ($P < 0.001$), gastric cancer ($P < 0.001$), colorectal cancer ($P < 0.001$) and hepatocellular carcinoma ($P = 0.002$). Meanwhile, the area under the ROC curve (AUC) value for miR-133a was 0.836, 0.888, and 0.99 in ESCC, gastric cancer and colorectal cancer. MiR-133a is a tumor suppressor with prognostic and diagnostic values for digestive system cancers. High miR-133a expression was associated with better prognosis and less adverse clinicopathological parameters. More research should be performed to test these findings.

Keywords MicroRNA-133a · Digestive system Cancer · TCGA · Meta-analysis

Abbreviations

miR-133a	MicroRNA-133a
HRs	hazard ratios
OR	Odds Ratio
95% CIs	95% confidence intervals
OS	overall survival
miRNAs	MicroRNAs
ESCC	esophageal squamous cell carcinoma
HCC	hepatocellular carcinoma
CNKI	China National Knowledge Infrastructure
CBM	China Biology Medicine disc
DFS	disease-free survival
TCGA	The Cancer Genome Atlas
AUC	the area under the ROC curve.

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Introduction

Digestive system cancers are among the ten most prevalent cancers worldwide, which also include esophageal squamous cell carcinoma (ESCC), gastric cancer, colorectal cancer, hepatocellular carcinoma (HCC), gallbladder cancer and pancreatic cancer [1]. As digestive system cancers are still the primary cause of cancer-related deaths, it is imperative to identify reliable diagnostic and prognostic biomarkers for digestive system cancers [2]. Additionally, revealing the association between valuable biomarkers and clinicopathological parameters can bring about a better understanding of digestive system cancers pathogenesis.

MicroRNAs (miRNAs) are small (19–24 nt), noncoding RNAs and are highly conserved. They have been observed to repress gene expression through the degradation of mRNAs after transcription [3]. MicroRNA-133a (miR-133a) is a multicopy gene locate on chromosomes 18 and 20 [4]. The impact of miR-133a on digestive system cancers has been explored in several cancers, including ESCC [5], gastric cancer [6], colorectal cancer [7] and pancreatic cancer [8]. However, the results are controversial and no meta-analysis has been conducted on miR-133a and its effect on the prognosis and clinicopathological parameters of digestive system cancers. We have conducted a meta-analysis on the impact of miR-133a on digestive system cancers, and verified the results through The Cancer Genome Atlas (TCGA).

Methods

Meta-Analysis

Search Strategy

Studies that previously investigated miR-133a and the prognosis or clinicopathological parameters of digestive system cancers were searched in PubMed, Web of Science, Science Direct, Cochrane Central Register of Controlled Trials, Wiley Online Library and Chinese Databases, including China National Knowledge Infrastructure (CNKI), China Biology Medicine disc (CBM), Chongqing VIP and Wan Fang Data (updated to 25th January 2018) with the following keywords: (miR-133a OR miRNA-133a OR microRNA-133a OR miR133a OR miRNA133a OR microRNA133a OR "miR 133a" OR "miRNA 133a" OR "microRNA 133a") and (malignan* OR cancer OR tumor OR tumour OR neoplas* OR carcinoma OR adenocarcinoma OR sarcoma) and (digestive OR gastrointestinal OR gastric OR stomach OR esophageal OR esophagus OR gut OR intestinal OR colorectal OR colonic OR rectal OR colon OR rectum OR Hepatocellular OR Hepatic OR Intrahepatic OR Liver OR gallbladder OR pancreatic OR pancrea*). Relevant meta-analyses, reviews and references cited in these papers were also assessed for potential studies. The searches were conducted by two reviewers independently, and any disagreement was resolved through discussion.

Inclusion and Exclusion Criteria

Eligible studies included in the meta-analysis met the following inclusion criteria: (1) study of patients with digestive system cancers; (2) detection of miR-133a expression in blood-based samples or primary tissue samples; (3) survival time or clinicopathological parameters were investigated based on miR-133a expression, and (4) raw data of clinicopathological parameter was available, or provided a hazard ratio (HR) or sufficient raw data (or Kaplan–Meier curve) to calculate HR. The exclusion criteria were as follows: (1) besides miR-133b, miR-133a was combined with other biomarker, and (2) unavailable HR or insufficient data (or Kaplan-Meier curve) to calculate HR, and unreported raw data of clinicopathological parameters.

Data Extraction and Study Quality Assessment

The relevant data were collected independently by two investigators. Moreover, all inconsistencies were resolved after discussion and a consensus was reached. The following information was extracted from each included study: first author, year of publication, country, type of cancer, stage, treatment, sample source, location of staining, test method, cut-off value, case, clinicopathological parameters, follow-up time, survival index, statistical method, HR as well as 95% CI, and the survival outcome of the high miR-133a expression group. When both univariate and multivariate analyses of survival index were available, the multivariate HR and 95% CI were used. If the Kaplan–Meier curve or sufficient raw data was the only available information, HR and 95% CI were calculated using the previously stated method [9]. The Newcastle-Ottawa quality assessment scale was applied to assess the quality of the study [10].

Statistical Analysis

The role of miR-133a in the prognosis of digestive system cancers was evaluated using pooled HRs with their 95% CIs. High expression of miR-133a was set as the case group, and HR < 1 with 95%CI not overlapping 1 was considered to implicate a better prognosis for the case group. Since the many heterogeneities among various studies resulted in a heterogeneity among individual HRs, we calculated a pooled HR using a random-effect model for all survival indices [11]. The association between miR-133a and clinicopathological parameters was assessed by calculating the Odds Ratio (OR) with 95%CI. Heterogeneity was assessed through the Q-statistic and I^2-statistic, $I^2 > 50\%$ was considered statistically significant and the random-effects model was chosen, otherwise the fixed-effects model was used. Publication bias was evaluated by Begg's test. Meta-analysis was conducted using the Stata 12.0 software (Stata Corporation, TX, USA), and P value<0.05 was considered to be statistically significant.

Analysis of TCGA Data

We downloaded the relevant data of miR-133a-3p (hereafter this text will be abbreviated as miR-133a) expression in digestive system cancers from TCGA database (https://cancergenome.nih.gov/). The expression and the values of

miR-133a were normally distributed, and the association between the clinicopathological parameters and miR-133a expression was assessed using the independent T test. The diagnostic capacity, sensitivity, and specificity of miR-133a for digestive system cancers was determined based on the AUC of the ROC curve, and the optimum diagnostic points were also calculated. Overall survival (OS) analysis was conducted using the Kaplan-Meier method and the log-rank test. Digestive system cancers patients were divided into high or low expression group according to the optimum diagnostic point. All statistical analyses were performed using the SPSS statistical software package, version 21.0 (IBM Corporation, Armonk, NY, USA), and $P < 0.05$ was considered statistically significant.

Results

Meta-Analysis

Literature Search, Study Characteristics and Quality Assessment

A search conducted on English databases and Chinese databases identified 139 relevant articles. After a review of titles and abstracts, 59 were found to be duplicated publications and 32 articles did not report miR-133a and digestive system cancers. Thus, 48 unique studies were screened for full text review, 16 studies were excluded as review or meta-analysis, and 19 trials were removed for not involving survival index or clinicopathological parameters. Meanwhile, upon further analysis of the remaining 13 potential trials, three articles without sufficient data were precluded. Finally, 10 publications with 1340 patients were included in the meta-analysis [5–8, 12–17]. Figure 1 showed the flow chart used for literature search. Among the included articles, nine articles were connected with survival index [5–8, 12–16] and four with clinicopathological parameters [5, 8, 14, 17]. With regard to the specific cancer type, four studies involved ESCC [5, 12–14], two studies investigated gastric cancer [6, 15], two studies involved colorectal cancer [7, 16], only one study involved pancreatic cancer [8] and HCC [17]. With regard to the survival index, eight studies assessed OS [5–8, 13–16] and two evaluated disease-free survival (DFS) [5, 12]. The following clinicopathological parameters with sufficient raw data were assessed: gender was involved in four studies [5, 8, 14, 17], four studies reported tumor differentiation [5, 8, 14, 17], lymph node metastasis was investigated in three trials [5, 8, 17] and TNM stage was investigated in four studies [5, 8, 14, 17]. The study characteristics and quality assessment results were summarized in Table 1 and Supplementary Table S1.

High miR-133a Expression Was Linked with Favorable OS

We evaluated eight studies which calculated OS based meta-analyses [5–8, 13–16], among them three studies involved ESCC [5, 13, 14], two studies investigated gastric cancer [6, 15], two studies involved colorectal cancer [7, 16] and one study investigated pancreatic cancer [8]. The result showed that an increased miR-133a expression was significantly linked with favorable OS in digestive system cancers (HR = 0.539, 95% CI: 0.416–0.698, $P < 0.001$, $I^2 = 23.4\%$) **(**Fig. 2a, Table 2**)**. Besides OS, the survival index of DFS in two ESCC studies [5, 12] was also assessed in the current meta-analysis, but no statistical significance was observed (HR = 0.832, 95% CI: 0.421–1.641, $P = 0.595$, $I^2 = 74.9\%$) **(**Fig. 2a, Table 2**).** According to statistical methods, we conducted further analysis on survival curve studies and HR was 0.561 (95% CI: 0.425–0.741, $P < 0.001$, $I^2 = 26.6\%$) **(**Fig. 2b, Table 2**)**. Meanwhile, digestive tract cancers including ESCC, gastric cancer and colorectal cancer were selected to performed subgroup analysis, and increased miR-133a expression also showed a correlation with improved OS in the pooled HR (HR = 0.558, 95% CI: 0.406–0.767, $P < 0.001$, $I^2 = 32.1\%$) **(**Fig. 2c, Table 2**)**. In addition, subgroup analysis was performed on specific cancer types including ESCC, gastric cancer and colorectal cancer, and pooled HRs in these three subgroups were found to be 0.427 (95% CI: 0.265–0.690, $P = 0.001$, $I^2 = 0\%$), 0.541(95% CI: 0.385–0.761, $P < 0.001$, $I^2 = 0\%$) and 0.798 (95% CI: 0.245–2.598, $P = 0.708$, $I^2 = 83.9\%$), respectively **(**Fig. 2d, Table 2**)**. While significant publication bias was found in the studies related to digestive system cancers ($P = 0.661$) **(**Fig. 3**)**.

The Association of miR-133a And Clinicopathological Parameters

With regard to digestive system cancers, an increased miR-133a expression was associated with negative lymph node metastasis and the OR was 0.219 (95% CI: 0.119–0.402, $P < 0.001$, $I^2 = 80.3\%$) (Supplementary Fig. S1A, Table 2). For tumor differentiation, well or moderate differentiation was significantly linked with high miR-133a expression (OR = 2.375, 95% CI: 1.489–3.789, $P < 0.001$, $I^2 = 75.5\%$) (Supplementary Fig. S1A, Table 2**)**. However, no statistically significant correlation was observed with gender and TNM stage; the pooled ORs were 0.958 (95% CI: 0.614–1.493, $P = 0.849$, $I^2 = 0\%$) and 0.599 (95% CI: 0.348–1.034, $P = 0.066$, $I^2 = 84.8\%$), respectively (Supplementary Fig. S1A, Table 2**)**. We also conducted meta-analysis on the clinicopathological parameters of ESCC, including gender, tumor differentiation and TNM stage, and the ORs were observed to be 1.212 (95% CI: 0.674–2.180, $P = 0.521$, $I^2 = 0\%$), 2.752 (95% CI: 1.495–5.065, $P = 0.001$, $I^2 = 87.70\%$) and 0.898

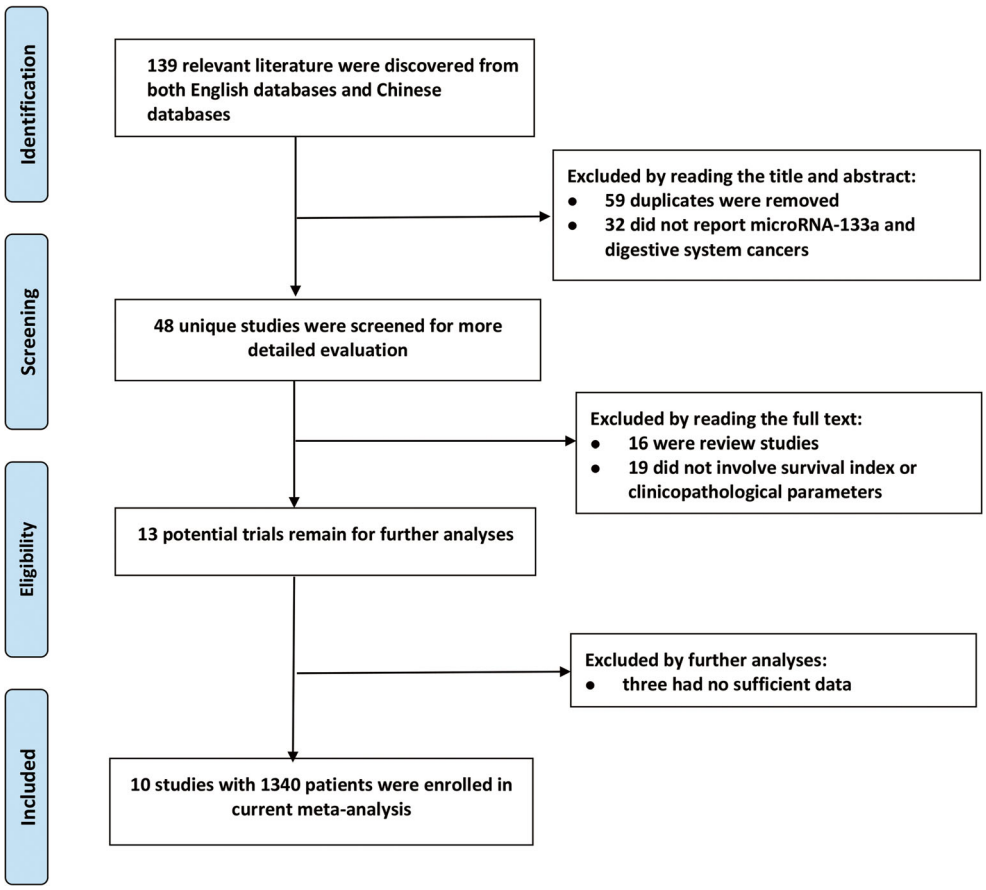

Fig. 1 The flow chart of literature research

(95% CI: 0.489–1.648, $P = 0.728$, $I^2 = 77\%$), respectively (Supplementary Fig. S1B, Table 2).

Analysis of TCGA Data

1285 digestive system cancers patients were included in the analysis. As shown in Table 3, the expression of miR-133a was significantly lower in cancer tissue compared with adjacent tissue for ESCC ($P < 0.001$), gastric cancer ($P < 0.001$), colorectal cancer ($P < 0.001$) and HCC ($P = 0.002$). MiR-133a expression was significantly linked with age ($P = 0.023$), lymph node metastasis ($P = 0.01$) and TNM stage ($P = 0.008$) of colorectal cancer, while no significant association was detected between clinicopathological parameters and other digestive system cancers. The ROC curves revealed the high diagnostic values of miR-133a for ESCC (AUC = 0.836), gastric cancer (AUC = 0.888) and colorectal cancer (AUC = 0.99). However, the diagnostic values of miR-133a for pancreatic cancer (AUC = 0.592) and HCC (AUC = 0.64) were limited (Fig. 4, Table 4). The optimum diagnostic points as well as their sensitivities and specificities for diagnosis of digestive system cancers were also shown in Table 4. However, no significant link between miR-133a and OS was detected for digestive system cancers, except for high expression of miR-133a was significantly linked with better prognosis for ESCC ($P = 0.037$) (Fig. 4, Table 4).

Discussion

MiRNAs participate in a series of critical processes of tumorigenesis, such as tumor cell mutation, proliferation, invasion, progression and metastasis [18]. Abnormal miRNAs expression have been observed in many studies, and miRNAs act as tumor suppressor or promoter in various cancers [19–21]. MiR-133a is one of the miRNAs as shown in many solid cancers, and most studies suggested that increased miR-133a was associated with a better OS [22–25]. Some metastasis-related oncogenes were observed to be regulated by miR-133a directly, such as CAV1 in head and neck squamous cell cancer [26], LASP1 in colorectal cancer [27], and FSCN1 in bladder cancer [28]. Among the eight studies which evaluated OS included in our meta-analysis, seven studies reported significant association between up-regulated miR-133a and favorable OS in digestive system cancers [5, 6, 8, 13–16], while one study reported the contrary [7]. Based on the pooled HR, our study successfully revealed that a high miR-133a expression in digestive system cancers was significantly linked with a favorable OS.

Table 1 The main characteristics and quality scores of the included studies

First author	Publication Year	Country	Cancer Type	Stage	Treatment	Sample source	Location of staining	Test method	Cut-off Value	Case (High/Low)		Follow-up (month)	Survival	Statistic method	HR	LL	UL	Outcome	NOS
Suzuki S [1]	2012	Japan	ESCC	I- IV	surgical treatment	Tissues	membrane	PCR	/	30	72	40	DFS	Univariate analysis	1.117	0.835	1.499	NS	6
Akanuma N [2]	2013	Japan	ESCC	I- IV	surgical treatment	Tissues	membrane, cytoplasm	PCR	0.012	62	22	120	OS	Survival curve	0.47	0.246	0.886	Better	6
Chen G [3]	2014	China	ESCC	I- IV	surgical treatment	Tissues	/	PCR	1.31	36	46	5–50	OS	Survival curve	0.459	0.268	0.752	Better	7
Wan TM [4]	2014	China	Colorectal cancer	I- IV	surgical treatment	Tissues	/	PCR	>2.5, <0.4	52	51	65	OS	Survival curve	1.488	1.005	4.38	Poor	7
Cheng Z [5]	2014	China	Gastric cancer	I- IV	surgical treatment	Tissues	membrane	PCR	/	26	150	60	OS	Survival curve	0.591	0.135	0.987	Better	5
Wang LL [6]	2014	China	Colorectal cancer	I-III	surgical treatment	Tissues	/	PCR	median level	84	85	80	OS	Survival curve	0.446	0.246	0.808	Better	7
Qin Y [7]	2014	China	Pancreatic cancer	I-II	surgical treatment	Tissues	membrane	PCR	median level	47	48	60	OS	Survival curve	0.475	0.296	0.761	Better	7
Gao SH [8]	2016	China	ESCC	I-III	surgical treatment	Tissues	/	PCR	/	62	64	60	OS	Multivariate analysis	0.3-68	0.1-64	0.7-73	Better	7
Gao SH [8]	2016	China	ESCC	I-III	surgical treatment	Tissues	/	PCR	/	62	64	60	DFS	Multivariate analysis	0.5-53	0.2-36	0.8-23	Better	7
Li CY [9]	2017	China	Gastric cancer	I- IV	surgical treatment	Tissues	/	PCR	/	105	256	70	OS	Survival curve	0.535	0.372	0.769	Better	6

Abbreviations: *ESCC* esophageal squamous cell carcinoma; /, not report, *OS* overall survival, *DFS* disease-free survival, *HR* hazard ratio, *LL* lower limit, *UL* upper limit; *: outcome was for high miR-133a expression; NOS, the scores of Newcastle-Ottawa quality assessment scale

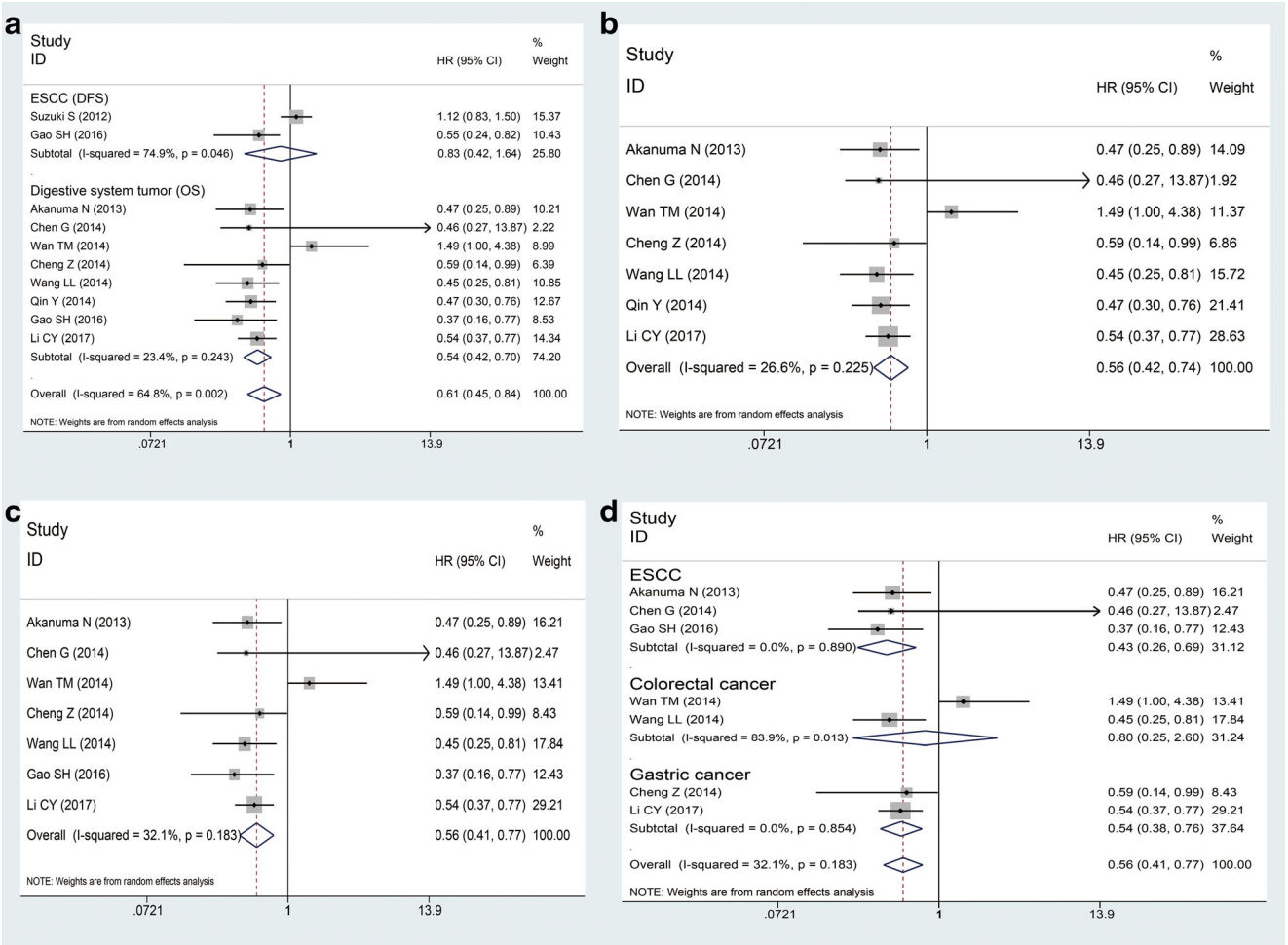

Fig. 2 The forest plots for the association between miR-133a expression and overall survival (OS) of digestive system cancers (**a**), disease-free survival (DFS) of esophageal squamous cell carcinoma (ESCC) (**a**), OS of digestive system cancers with survival curve (**b**), OS of digestive tract cancers (**c**), OS of ESCC, gastric cancer and colorectal cancer (**d**)

There were several advantages in our meta-analysis compared with the previous meta-analysis [29] that included various solid cancers, and in which the digestive system cancers were just set as a subgroup. First of all, the included studies and cases extended from five studies with 533 cases to 10 studies with 1340 cases. Secondly, we operated several subgroup analyses to further explore the role of miR-133a in digestive system cancers. After the only one study which evaluated OS with multivariate analysis was removed [5], significant association between high miR-133a expression and better OS was also achieved in survival curve studies. Meanwhile, we operated a subgroup analysis on digestive tract cancers after precluding one pancreatic cancer study [8], and the same conclusion was also achieved. These results indicated that the association between miR-133a and the prognosis of digestive system cancers was reliable in our meta-analysis. Thirdly, gastric cancer studies [6, 15] were incorporated into our meta-analysis which were lacking in the previous meta-analysis. Subgroup analysis was also performed on specific cancers, including ESCC, gastric cancer and colorectal cancer. Increased miR-133a expression was correlated with better OS also observed in ESCC and gastric cancer, while no significant association between high miR-133a and better OS was detected in colorectal cancer. Wang L.L. et al. demonstrated that colorectal cancer patients with high miR-133a expression had better OS [16], while Wan T.M. et al. reported that increased miR-133a was correlated with adverse clinical characteristics and poorer OS [7]. Thus, more research should be conducted to verify these controversial results due to the limited studies and sample size. Fourthly, for ESCC, the survival index of DFS was also included to conduct meta-analysis, while favorable DFS was not detected in the high miR-133a expression group. Fifthly, the impact of miR-133a on clinicopathological parameters of digestive system cancers was also investigated in our study, and increased miR-133a expression was associated with negative lymph node metastasis and better tumor differentiation. We also performed further analysis on clinicopathological parameters of ESCC studies. Likewise,

Table 2 Summarized HRs and ORs in this meta-analysis

Group	Number of studies	Number of patients	HR/OR (95% CI)	P value	Heterogeneity test		Model
					I^2 (%)	P value	
Digestive system tumor (OS) #	8	1196	0.539(0.416–0.698)	<0.001	23.40%	0.243	Random effect model
Digestive tract cancers (OS) #	7	1101	0.558(0.406–0.767)	<0.001	32.10%	0.183	Random effect model
ESCC (OS) #	3	292	0.427(0.265–0.690)	0.001	0.00%	0.89	Random effect model
Gastric cancer (OS) #	2	537	0.541(0.385–0.761)	<0.001	0.00%	0.854	Random effect model
Colorectal cancer (OS) #	2	272	0.798(0.245–2.598)	0.708	83.90%	0.013	Random effect model
ESCC (DFS) #	2	228	0.832(0.421–1.641)	0.595	74.90%	0.046	Random effect model
Survival curve (OS) #	7	1070	0.561(0.425–0.741)	<0.001	26.60%	0.225	Random effect model
Gender (DSC)	4	343	0.958(0.614–1.493)	0.849	0.00%	0.431	Fixed effect model
Tumor Differentiation (DSC)	4	343	2.375(1.489–3.789)	<0.001	75.50%	0.007	Random effect model
Lymph node metastasis (DSC)	3	261	0.219(0.119–0.402)	<0.001	80.30%	0.006	Random effect model
TNM stage (DSC)	4	343	0.599(0.348–1.034)	0.066	84.80%	0.001	Random effect model
Gender (ESCC)	2	208	1.212(0.674–2.180)	0.521	0.00%	0.602	Fixed effect model
Tumor Differentiation (ESCC)	2	208	2.752(1.495–5.065)	0.001	87.70%	0.004	Random effect model
TNM stage (ESCC)	2	208	0.898(0.489–1.648)	0.728	77%	0.037	Random effect model

Abbreviations: *ESCC* esophageal squamous cell carcinoma, *DSC* digestive system tumor, *HR* hazard ratio, *OR* odds ratio, *OS* overall survival, *DFS* disease-free survival; #, *HR* the remaining results were ORs

high expression of miR-133a was linked with better tumor differentiation.

In addition, we also gathered patient information from TCGA database to further verify the impact of miR-133a on digestive system cancers. The expression of miR-133a was significantly lower in cancer tissue compared with adjacent tissue for ESCC, gastric cancer, colorectal cancer and HCC, and miR-133a expression shown a high diagnostic value for ESCC, gastric cancer and colorectal cancer. The various sources of heterogeneity in TCGA data did not clear, such as sample sources, test method, location of staining and treatment of patients. Additionally, publication bias, heterogeneity source analysis, sensitivity analysis and regression analysis cannot be conducted on TCGA data to guarantee the accuracy and stability of pooled results. So, we did not combine ESCC, gastric cancer, colorectal cancer, pancreatic cancer and HCC to evaluate the impact of miR-133a on the prognosis of digestive system cancers. Meanwhile, ESCC, gastric cancer and colorectal cancer were also not combined to assess the association of miR-133a and the prognosis of digestive tract cancers. We only analyzed the association of miR-133a and specific cancers, significant link between miR-133a and OS was only detected for ESCC, but not for gastric cancer, colorectal cancer, pancreatic cancer and HCC. As mentioned above, the results of TCGA data were similar to the meta-analysis of specific cancer subgroups. However, considering TCGA data with many sources of heterogeneity and the limited cases, more research should be performed to verify our results.

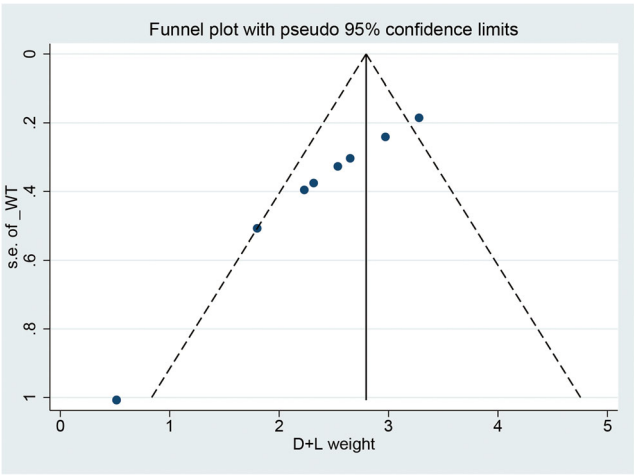

Fig. 3 Begg's funnel plot for publication bias on digestive system cancers studies

The miR-133 family includes miR-133a and miR-133b, similar to miR-133a, aberrant miR-133b expression has been demonstrated in various cancers [30–32]. Meanwhile, studies have reported that both miR-133a and miR-133b might all serve as tumor suppressors and enroll in the invasion and metastasis of various solid cancers [33–35]. Moreover, targeting of FSCN1 by both miR-133a and miR-133b in ESCC has been observed. This finding could possibly be reflected in other cancers as they differed by only one nucleotide, and thus they might share many potential target genes [7, 34]. Together with the evidences of our study, all these results demonstrated that miR-133 family might act as a reliable independent diagnostic as well as prognostic biomarker, and digestive system cancers, even other cancers with high

Table 3 Association between miR-133a-3p expression and clinicopathological parameters of digestive system cancers (TCGA data)

Variables	ESCC				Gastric cancer				Colorectal cancer				Pancreatic cancer				HCC			
	Case	Expression		P	Case	Expression		P	Case	Expression		P	Case	Expression		P	Case	Expression		P
		Mean	SD			Mean	SD			Mean	SD			Mean	SD			Mean	SD	
Tissue																				
Cancer tissue	156	4.12	1.84	<0.001	376	4.38	1.85	<0.001	322	4	1.58	<0.001	71	0.38	0.25	0.68	361	2.09	1.22	0.002
Adjacent tissue	8	6.88	1.97		39	7.38	1.46		9	8.64	1.34		3	0.44	0.29		49	2.47	0.71	
Age																				
<60 years	73	3.91	1.93	0.171	117	4.63	2.02	0.096	115	4.25	1.56	0.023	24	0.35	0.19	0.517	167	2.21	1.33	0.07
≧60 years	83	4.31	1.76		256	4.28	1.77		206	3.84	1.55		47	0.39	0.28		194	1.98	1.1	
Gender																				
Male	23	3.82	1.83	0.4	130	4.49	1.92	0.408	150	4.05	1.54	0.533	39	0.36	0.25	0.611	118	2.14	1.31	0.56
Female	133	4.18	1.85		246	4.32	1.82		171	3.94	1.58		32	0.39	0.25		243	2.06	1.17	
T																				
T1-T2	67	4.03	1.8	0.565	93	4.21	1.8	0.314	54	3.88	1.33	0.579	13	0.29	0.15	0.191	267	2.09	1.17	0.733
T3-T4	89	4.2	1.88		283	4.44	1.87		266	4.01	1.61		57	0.39	0.27		92	2.04	1.32	
N																				
N0	73	4.26	1.93	0.469	115	4.22	2.06	0.343	175	3.79	1.55	0.01	21	0.38	0.20	0.922	248	2.04	1.27	0.807
N1	81	4.04	1.76		254	4.41	1.75		146	4.23	1.54		47	0.37	0.27		4	2.21	0.93	
M																				
M0	132	4.11	1.89	0.503	338	4.39	1.84	0.549	225	3.92	1.59	0.096	26	0.33	0.24	0.586	261	2.09	1.28	0.979
M1	9	4.55	1.39		22	4.15	2.13		42	4.36	1.38		3	0.25	0.05		4	2.07	1.22	
TNM stage																				
Stage I-II	92	4.15	1.94	0.842	167	4.32	1.95	0.546	167	3.77	1.58	0.008	64	0.39	0.25	0.159	249	2.09	1.19	0.724
Stage III-IV	64	4.09	1.71		201	4.44	1.78		148	4.24	1.53		6	0.24	0.05		89	2.03	1.33	

Abbreviations: *TCGA* The Cancer Genome Atlas, *ESCC* esophageal squamous cell carcinoma, *HCC* hepatocellular carcinoma

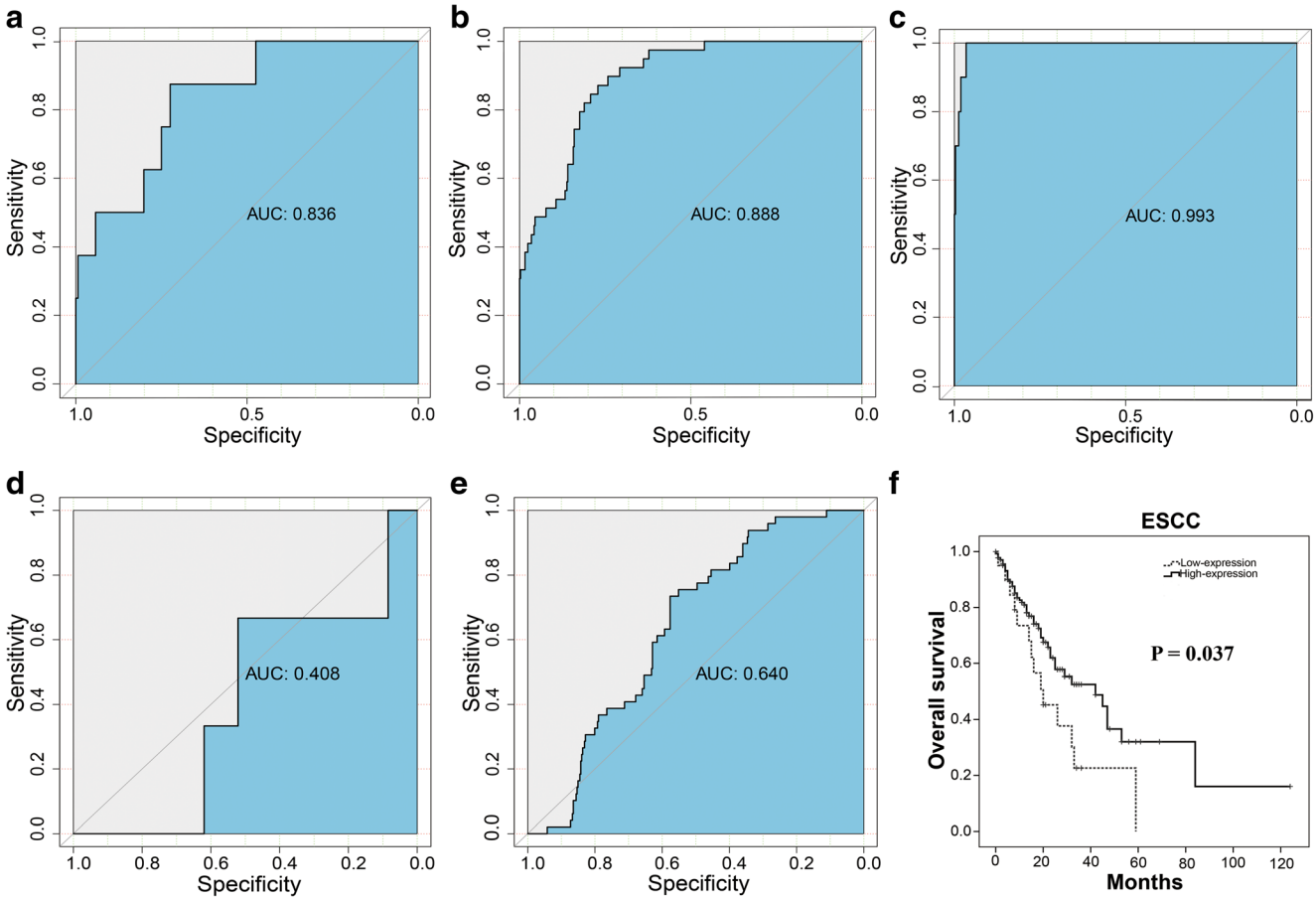

Fig. 4 The ROC curves of miR-133a-3p for esophageal squamous cell carcinoma (ESCC) (**a**), gastric cancer (**b**), colorectal cancer (**c**), Pancreatic cancer (**d**) and Hepatocellular cancer (HCC) (**e**); The Kaplan-Meier survival curve for miR-133a-3p and ESCC (**f**)

expression of miR-133 might have a favorable prognosis and less adverse clinicopathological parameters. Although these findings implicated a possible role of miR-133 family in cancers, the exact mechanism remains to be elucidated. Hence, studies on this topic are of significant importance.

Table 4 Summarized results in the analysis of TCGA data

Cancer	ROC curve					Survival analysis		
	AUC	P[a]	The optimum diagnostic point	Sensitivity (%)	Specificity (%)	High-expression (N)	Low-expression (N)	P[b]
ESCC	0.836 (0.71–9.961)	0.001	5.475	87.5	72.4	136	20	0.037
Gastric cancer	0.888 (0.844–0.932)	<0.001	5.91	87.2	77.1	86	290	0.121
Colorectal cancer	0.99 (0.98–1.0)	<0.001	6.893	100	96.3	11	310	0.473
Pancreatic cancer	0.592 (0.314–0.869)	0.593	0.254	100	38.0	44	27	0.313
HCC	0.64 (0.574–0.707)	0.001	2.162	73.5	57.6	154	207	0.85

Abbreviations: *TCGA* The Cancer Genome Atlas, *ESCC* esophageal squamous cell carcinoma, *HCC* hepatocellular carcinoma, *AUC* the area under the ROC curve

[a] P value for AUC

[b] P value for Kaplan-Meier survival curve

Our study has several limitations which are listed as follows. Firstly, obvious heterogeneities were detected in the meta-analysis, the potential sources of heterogeneity might be as follows: the controversial results among included studies; the combined pooled HR resulting in a critical bias due to the application of different statistical methods in different studies, including survival curve, univariate and multivariate analysis; lower accuracy of HRs obtained from univariate analysis and survival curves than those from multivariate analysis which accounted for intermixed factors were accounted in multivariate analysis; inevitable errors due to HRs acquired from survival curves despite repeated data extraction. Secondly, data can only be gained from the publications directly, we were unable to acquired specific raw data of patients from authors. This impeded us from conducting further analysis on some included studies, especially clinicopathological parameters studies. Thirdly, the countries of enrolled studies in current meta-analysis were only involved China and Japan, and our study should be confirmed by more trials from other countries. Fourthly, only ten trials with 1340 patients were involved in our studies to assess the role of miR-133a in digestive system cancers, especially for clinicopathological parameters with only four studies and 343 cases. Fifthly, miR-133a expression was only detected in primary cancer tissue, which cannot acquire a less invasive and easy approach of sampling and promote early diagnosis as well as prognosis. Last but not least, all enrolled studies were retrospective trails, which tended to be published when positive results were demonstrated. Thus, the impact of miR-133a on prognosis and clinicopathological parameters of digestive system cancers might be overrated.

In the light of our findings, we would like to propose several recommendations for future research: First of all, more research should explore blood-based miR-133a rather than only focusing on primary tissue. Secondly, the impact of miR-133a on digestive system cancers should be paid more attention, thus enlarging the literature and tackling the inconsistent reports, especially research beyond China and Japan. Thirdly, miR-133a should be combined with miR-133b, namely, the miR-133 family should be totally investigated to better understand their functions in digestive system cancers. Furthermore, large scale prospective studies with long-term follow-up must be conducted, and providing detailed descriptions of patients to allow future analyses could be enabled, such as the raw data of patients and specific treatment. Lastly, multivariate analysis should be applied for survival analysis.

Conclusion

In conclusion, our study revealed that miR-133a is a tumor suppressor with diagnostic and prognostic values for digestive system cancers. High miR-133a expression was associated with better prognosis and less adverse clinicopathological parameters. More research should be performed to test these findings.

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Compliance with Ethical Standards

Conflict of Interest Statement The authors declare no conflict of interest.

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