



Identification of miR-375 as a potential prognostic biomarker for esophageal squamous cell cancer: A bioinformatics analysis based on TCGA and meta-analysis

He-San Luo^{a,b}, De-Hua Wu^{a,*}

^a Department of Radiation Oncology, Nanfang Hospital, Southern Medical University, Guangzhou, 510515, China

^b Department of Radiotherapy, Shantou Central Hospital, Shantou, 515031, China

ARTICLE INFO

Keywords:

miR-375
TCGA
Bioinformatics analysis
Esophageal squamous cell cancer

ABSTRACT

Accumulating evidence has demonstrated that aberrantly expressed miRNAs in cancer tissues regulated various cellular processes related to carcinogenesis. The present study aimed to identify the differentially expressed miRNAs between esophageal squamous cell cancer (ESCC) and adjacent normal esophageal tissue (ANET). In our present study, we identified 129 differentially expressed miRNAs between ESCC and ANET by analyzing high-throughput miRNA data downloaded from TCGA database. After investigating the prognostic value of the 129 differential expressed miRNAs, eight miRNAs were found to be associated with prognosis of patients with ESCC. The clinical significance and bio-function of miR-375 was further examined. We performed Gene Set Enrichment Analysis (GSEA) to identify the top three gene sets that significantly altered between the patients with miR-375 low expression and high expression. In order to explore the mechanism of the development and progression of ESCC, the role of miR-375 in ESCC and its four candidate target genes was examined. At last, we performed a meta-analysis to verify the prognostic value of miR-375 in ESCC. In conclusion, our findings suggest that miR-375 serves as a promising independent prognostic factor for ESCC and function as a tumor suppressor.

1. Introduction

According to the world cancer statistics, esophageal cancer (EC) is one of the most common malignant tumors in the world. In China, ESCC(esophageal squamous cell cancer),the main pathological type of EC, is the fourth most common cause of cancer-related deaths, with an estimated 223,000 new cases and 197,200 deaths annually [1]. Although improvement have been made in prognosis of ESCC due to multidisciplinary treatment and early diagnostic technology, the survival rate remains unsatisfactory, with an approximately 17–20% overall 5-year survival rate [2]. Moreover, as many as 35–60% of patients with local advanced ESCC who underwent preoperative chemoradiotherapy and surgery relapsed within 2–3 years [3]. The patients experienced recurrent disease after chemo-radiotherapy have very poor prognosis due to lack of effective therapy. Thus, early diagnosis, prognosis prediction and effective treatment are the key factors to improve prognosis of ESCC.

MicroRNAs (miRNAs), a key component of the noncoding RNA family, are consisting of approximately 18–25 nucleotides, which involved in the post-transcriptional regulation of gene expression, leading

to degradation of mRNA and inhibition of their translation [4]. Aberrant expressions of miRNAs had been reported in many types of solid malignant tumors, functioning either as oncogene [5] or tumor suppressors [6]. Accumulating evidence had demonstrated that aberrantly expressed miRNAs in cancer tissues regulated various carcinogenesis processes including cell proliferation, migration, invasion, and metastasis [7–9], indicating a large potential to serve as promising biomarkers in the early diagnosis, prognosis prediction, and effective personalized targeted therapies [5].

However, there exists inconsistency in previous studies because of small sample size, different technological platforms, and different methods for processing and analysis. To overcome these problems, a more stable and consistent high-throughput data is necessary. The Cancer Genome Atlas Project (TCGA) is a National Cancer Institute effort to profile at least 20 different tumor types using genomic platforms and to make raw and processed data available to all researchers [10]. To our joy, the TCGA released a large number of miRNA sequencing and RNA sequencing data for esophageal cancer patients. In our present study, we performed an analysis using the high-throughput miRNA data downloaded from TCGA database to identify the

* Corresponding author.

E-mail address: wudehua1961@163.com (D.-H. Wu).

<https://doi.org/10.1016/j.prp.2019.01.009>

Received 1 November 2018; Received in revised form 18 December 2018; Accepted 5 January 2019

0344-0338/ © 2019 Elsevier GmbH. All rights reserved.

differentially expressed miRNAs between ESCC and ANET. Additionally, we batch investigated the prognostic value of the differential expressed miRNAs and identified four miRNAs that could predict patients' prognosis. As a result, miR-375 was selected for further investigation. Furthermore, we performed Gene Set Enrichment Analysis (GSEA) to identify key pathways and gene sets that altered between the patients with miR-375 low expression and high expression. The prognostic value of miR-375 was confirmed by a meta-analysis. At last, we focused on the role of miR-375 and its candidate target gene ARL4C, which may provide new insight into understanding the mechanism of the development and progression of ESCC.

2. Materials and methods

2.1. Data processing and Screening of differentially expressed miRNAs and RNAs

The raw sequencing data (RNA-sequencing and miRNA-sequencing) and clinical information involved in this study were downloaded from an online TCGA data platform FireBrowse (<http://firebrowse.org/>). A dataset contained 185 esophageal carcinoma tissues and 13 matched normal tissues was selected for further investigation, which included 1046 miRNA expression values and 29699 RNA expression values. After well chosen, a total of 95 ESCC tissues and 13 ANET were enrolled in this study. R language packages were applied for the miRNA sequencing data and RNA sequencing data processing. Firstly, these raw sequencing data were standardized, and then the miRNAs or RNAs with more than 50% expression values zero were removed. The differentially expressed miRNAs between ESCC and ANET were analyzed using limma package in R. The fold changes (FCs) in the expression of individual miRNA were calculated and differentially expressed miRNAs with $\log_2|FC| > 2.0$ and $P < 0.05$ were considered to be significant. Similarly, the same analysis was performed for the RNA sequencing data.

2.2. Identification of prognostic miRNAs differentially expressed in ESCC

The differentially expressed miRNA profile was standardized by \log_2 transformed. The prognostic value of each differentially expressed miRNA was batch evaluated using dplyr and survival package in R. The miRNAs those were significantly associated with overall survival were identified as prognostic miRNAs (threshold of $P < 0.05$ for the log rank test).

2.3. The prediction of target genes for prognostic miRNAs

The target genes of prognostic miRNAs were predicted using TargetScan (<http://www.targetscan.org/>), miRDB (<http://www.mirdb.org/mirDB/>), and miRanda (<http://www.microrna.org/>) online analysis tools. To improve the bioinformatics analysis reliability, the overlapping genes between above target genes and differentially expressed genes were defined as the candidate target genes of the prognostic miRNAs using Venn diagram.

2.4. GSEA analysis

GSEA is a computational methodology to identify key pathways and gene sets that enriched in a large set of genes between two biological states, such as disease phenotypes [11]. This analysis was done using GSEA software 2.2.3. The Molecular Signatures Database (MSigDB) C2 collection, consisting of canonical pathways and experimental signatures curated from publications was used for the analysis.

2.5. Meta-analysis

This meta-analysis was carried out according to the guidelines of meta-analysis of observational studies in epidemiology (MOOSE).

Studies were performed by searching PubMed, Embase, and Cochrane Library. Eligible studies enrolled in this meta-analysis should meet the following criteria: I) Including the patients histologically diagnosed as ESCC; II) reporting explicit methods for the detection of miR-375 expression in tumor tissue or blood; III) investigating the association between miR-375 expression and survival outcome; IV) reporting sufficient data to estimate the hazard ratio (HR) and 95% confidence intervals (CI) according to miR-375 expression. The data from all eligible studies were extracted. The high or low expression of miR-375 was defined according to the cut-off values provided by the authors. HRs and their 95% CIs were combined to evaluate the association between miR-375 expression and prognosis. If the statistical variables were described in the study, we pooled them directly. Otherwise, the statistical variables were calculated from an available numerical data in the articles according to the methods described by Tierney et al [12]. An observed HR greater than 1 indicated a better prognosis in patients with miR-375 high expression. Statistical heterogeneity was assessed by visual inspection of forest plots, by performing the Chi-square test (assessing the I^2 value) and calculating the I^2 statistic. If the I^2 value was less than 0.05 and/or I^2 exceeded 50%, indicating the presence of heterogeneity, a random-effects model (the DerSimonian-Laird method) was used. Otherwise, the fixed-effects model (the Mante-Haenszel method) was used. All analyses were performed using Review Manager Version 5.3, a P value less than 0.05 was considered statistically significant.

2.6. Statistical analysis

The data were expressed as mean \pm standard deviation (SD). The expression levels of miRNAs in ESCC and ANET were analyzed using unpaired t -test The chi-square test and the t were used to assess the relationship between miRNA expression and clinical features. Kaplan-Meier survival analysis and univariate/multivariate Cox proportional hazard regression analysis were used to analyze the relationship between each miRNA (low vs. high level) or prognostic miRNA signature (low vs. high risk) and prognosis. A P value less than 0.05 was considered statistically significant. The statistical analysis was performed using IBM SPSS Statistics software program version 22.0 (IBM Corp., NY, USA).

3. Results

3.1. Identification of differentially expressed miRNAs in ESCC

Ninety-five of the 184 patients with esophageal cancer, who were pathologically diagnosed ESCC, were finally enrolled in our study. The detailed clinical characteristics included age at diagnosis, gender, T stage, N stage, M stage, and TNMstage (Table 1). Differentially

Table 1
Clinical character of patients with ESCC.

Variables	Total
Gender	
Male	79(84%)
Female	15(16%)
Age	
$\leq 65y$	73(77.7%)
$> 65y$	21(22.3%)
T stage	
T1 + T2	40(42.6%)
T3 + T4	54(57.4%)
N stage	
N0	54(57.4%)
N1-3	38(40.5%)
Nx	2(2.1%)
M stage	
M0	89(94.6%)
M1	5(5.3%)
Stage	
Unknown	2(2.1%)
I + II	61(64.9%)
III + IV	31(33.0%)

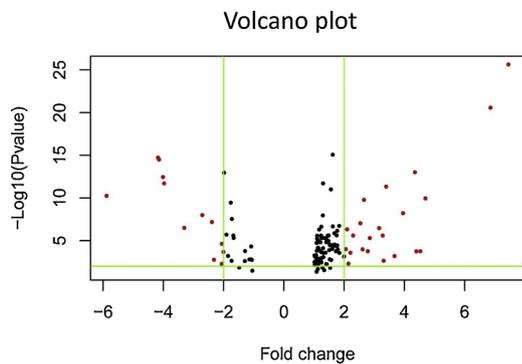


Fig. 1. Volcano plot of differentially expressed miRNAs. The dot represent the differentially expressed miRNAs between ESCC and normal matched normal tissues, and the red one represent the $|\log_2FC| > 2.0$ (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

expressed miRNAs between ESCC and ANET were identified after analysis using limma package in R. According to the cut-off criteria ($P < 0.05$ and $|\log_2FC| > 1.0$), 129 differentially expressed miRNAs including 101 up-regulated and 28 down-regulated miRNAs were identified (data not shown). In order to prove the P value and $|\log_2FC|$ whether conform to logic with different-test, we presented the result as Volcano plot (Fig.1). Unsupervised hierarchic cluster analysis revealed that ESCC were different from ANET based on differentially expressed miRNAs patterns (data not shown).

3.2. Identification of prognostic miRNAs differentially expressed in ESCC

To identify the differentially expressed miRNAs that would be associated with the outcomes of the patients with ESCC, we performed a batch analysis to evaluate the prognostic values of the differentially expressed miRNAs using dplyr and survival package in R. Showed as Table 2, eight differentially expressed miRNAs were found to be correlated with the overall survival (OS). Of the eight miRNAs, miR-375 was selected for further investigation. The results showed that patients in high expression of miR-375 group had poorer prognosis than those in low expression group (Fig.2). Showed as Table 3, miR-375 expression was significantly associated with the gender ($p = 0.049, \chi^2 = 3.887$) but not with other clinical characteristic. Taking into account the following clinical characteristic: age, gender, lymph node stage, stage, T stage, M stage and TNM stage, univariate and multivariate Cox regression analysis were performed to test the effect of the miR-375 (low expression vs. high expression) on OS. In univariate analysis, age (HR = 2.931, $P = 0.041$), N stage (HR = 3.192, $P = 0.009$) and TNM stage (HR = 2.639, $P = 0.034$) were associated with OS in ESCC patients. In multivariate analysis, all the variables $p < 0.10$ were selected for further analysis. Age (HR = 6.727, $P = 0.002$), N stage (HR = 2.931, $P = 0.024$) and the miR-375 expression (HR = 0.353,

Table 2
Eight DEMs correlated with overall survival.

miRNAs	logFC	AveExpr	t	P.Value	adj.P.Val	B
hsa-mir-767	4.397771	2.772196	4.327683	3.30E-05	0.000179	1.569273
hsa-mir-105-1	3.671912	2.2529	3.953602	0.000136	0.000644	0.225418
hsa-mir-105-2	3.312169	2.24459	3.527051	0.000611	0.002291	-1.19373
hsa-mir-219-1	1.09322	1.903992	3.791943	0.000243	0.001078	-0.32715
hsa-mir-3662	1.086902	-0.00083	2.357549	0.020139	0.045069	-4.38413
hsa-mir-382	1.038654	4.06603	3.889787	0.000171	0.000796	0.0052
hsa-mir-338	-1.98546	8.73764	-9.26422	1.71E-15	1.12E-13	24.72011
hsa-mir-375	-5.88429	9.09068	-7.9562	1.60E-12	5.69E-11	17.9775

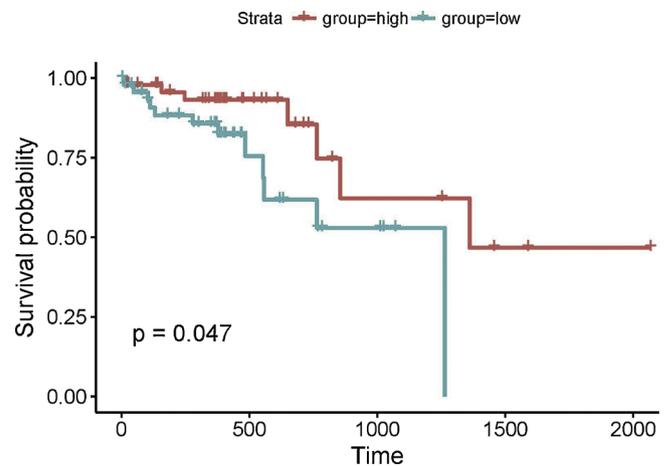


Fig. 2. Kaplan-Meier curve for the miR-375 expression in patients with ESCC. The patients were stratified into high group and low group based miR-375 expression.

Table 3
Association of miR-375 and clinical features.

Variables	Total	miR-375 expression		P value	
		Low	High		
Gender	Male	79(84%)	43	36	0.049
	Female	15(16%)	4	11	
Age	≤ 65y	73(77.7%)	38	35	0.458
	> 65y	21(22.3%)	9	12	
T stage	T1 + T2	40(42.6%)	18	22	0.404
	T3 + T4	54(57.4%)	29	25	
N stage	N0	54(57.4%)	29	25	0.397
	N1-3	38(40.5%)	17	21	
M stage	M0	89(94.6%)	45	44	0.646
	M1	5(5.3%)	2	3	
Stage	I + II	61(64.9%)	33	28	0.270
	III + IV	31(33.0%)	13	18	

$P = 0.047$) were showed to be independent prognostic factors in ESCC patients (Table 4).

3.3. Candidate target genes prediction of miR-375

We next investigated the differentially expressed genes (DEGs) between ESCC and ANET using limma package in R. According to the cut-off criteria ($P < 0.05$ and $|\log_2FC| > 1.0$), 5580 differentially expressed RNAs were identified. In order to prove the p value and $|\log_2FC|$ whether conform to logic with different-test, we presented the result as Volcano plot (Fig. 3A). To identify the target genes of miR-375, TargetScan, miRDB, and miRpathDB online analysis tools were applied

Table 4
Univariate and multivariate Cox regression analysis in ESCC patients.

Variables	Univariate analyses		Multivariate analyses	
	HR(95%CI)	P value	HR(95%CI)	P value
Gender(male vs female)	0.140(0.0181-0.70)	0.058	0.318(0.039-2.607)	0.286
Age(≤ 65y vs > 65y)	2.931(1.046-8.223)	0.041*	6.727(1.993-22.703)	0.002*
T stage(T1/2 vs T3/4)	1.961(0.744-5.174)	0.173	1.840(0.602-5.622)	0.284
N stage(N0 vs N+)	3.192(1.344-7.583)	0.009*	3.472(1.179-10.229)	0.024*
M stage(M0 vs M1)	3.450(0.893-12.108)	0.053	3.424(0.747-15.698)	0.113
Stage(I/II vs III/IV)	2.639(1.076-6.470)	0.034*	1.105(0.455-2.682)	0.826
miR-375(low vs high)	0.380(0.141-1.025)	0.056	0.353(0.126-0.987)	0.047*

* Indicate p < 0.05.

to predict the candidate target genes. Then the overlapping genes among the three tools and the DEGs were presented in Venn plot as Fig. 3B and Table 5, in which the four overlapping candidate genes of miR-375 were identified.

3.4. Pathways and gene set enrichment analysis in patients with miR-375 low expression and high expression

To further investigate the bio-function of miR-375 in ESCC; we used Gene Set Enrichment Analysis (GSEA) to identify key pathways and gene sets that altered between the patients with miR-375 low

expression and high expression. As a result, 180 gene sets were up-regulated in the miR-375 high expression group, among which three gene sets were significant enriched at false discovery rate (FDR) < 0.25 and three gene sets were significant enriched at a nominal p-value < 0.05. 1567 gene sets were regulated in the miR-375 low expression group, but no gene sets were significant enriched at FDR < 0.25. The three gene sets are shown for in Tables 6 and Fig. 4 based on the normalized enriched score (NES). Interestingly, all the three gene sets enriched in the miR-375 high expression group were involved in the RNA polymerase I transcriptional activity.

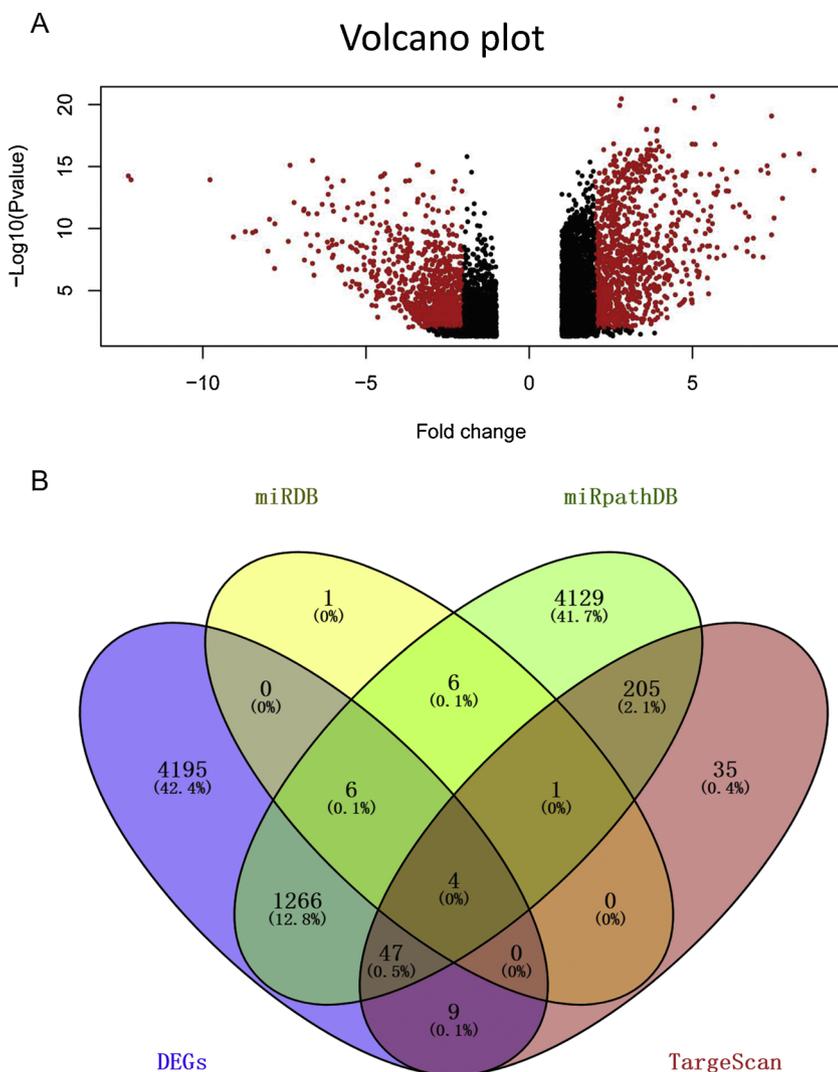


Fig. 3. Identification of differentially expressed RNAs and Candidate target genes prediction of miR-375. (A) Volcano plot of differentially expressed RNAs between ESCC and normal matched normal tissues, the red dot represent differentially expressed RNAs with |log2FC| > 2.0. (B) The overlapping target genes were predicted using TargetScan, miRDB, miRpathDB online analysis tools and the DEGs (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

Table 5
Candidate target genes of miR-375.

Symbol Genes	logFC	AveExpr	t	P.Value	adj.P.Val	B
POU3F1	2.894233	-0.13805	4.168548	6.23E-05	0.000329	1.147178
ARL4C	2.315671	4.017962	8.106328	9.13E-13	4.69E-11	18.63853
ISL2	1.118251	-1.12829	2.821019	0.005705	0.016142	-3.05786
HNF1B	-5.73725	-4.70141	-6.29134	7.00E-09	1.13E-07	9.907428

Table 6
Top 3 gene sets enriched in miR-375 high expression.

NAME	SIZE	ES	NES	NOM p-val	FDR q-val
REACTOME_RNA_POL_I_TRANSCRIPTION	75	-0.71	-1.99	0.002	0.164
REACTOME_RNA_POL_I_PROMOTER_OPENING	51	-0.82	-1.97	0.002	0.105
REACTOME_RNA_POL_I_RNA_POL_III_AND_MITOCHONDRIAL_TRANSCRIPTION	108	-0.58	-1.84	0.013	0.239

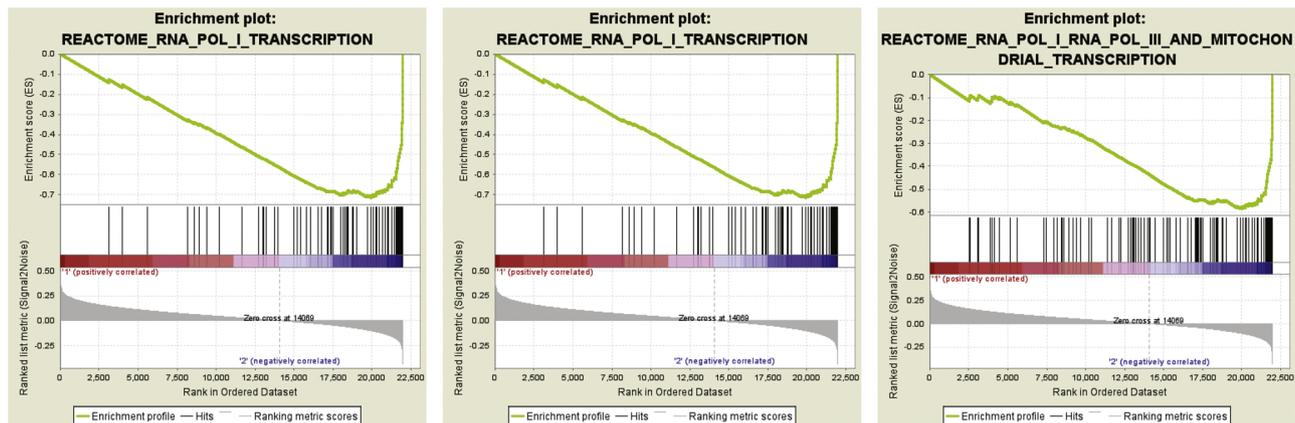


Fig. 4. The three gene sets enriched in the miR-375 high expression subtype.

3.5. Meta-analysis of prognostic significance of miR-375 in ESCC

A meta-analysis was performed to clarify the prognostic role of miR375 in the ESCC. Thirty-two records for miR-375 and esophageal cancer were obtained from a primary literature search in PubMed, EMBASE and web of science. After manually screening, eight eligible studies were chosen for this systematic review (Table 7). 1230 patients

with ESCC from United States, Canada, Japan, Chinese, Denmark and German were enrolled in this meta-analysis. A fixed model was used since there was no heterogeneity among the nine cohorts ($I^2 = 33\%$, $P = 0.16$) and the pooled HR was 1.69 (95% CI: 1.47–1.95, $P < 0.001$), as shown by Fig. 5, Pooled HR > 1.0 indicated that high expression of miR-375 may be associated with well OS in ESCC. The funnel plot was used to evaluate the publication bias. The funnel plot

Table 7
Characteristics of all studies included in the meta-analysis.

Study	Origin of population	Tumor type	Age(years,n)	Gender (M/F)	Sample (number)	Stage	Follow-up (months)	Source of miRNA	MiR-375 assay	Cut-off definition	Survival analysis	HR
2011 Oesophagus [26]	China	ESCC	Mean 66	M 43 F 17	60	I-IV	Over 60	Tissue	qRT-PCR	Normal	OS, DFS	DE
2012 EOBT [22]	Kyoto	ESCC	< = 65y, 25 > 65y, 25	M 44 F 6	50	0-IV	36	Plasma	qRT-PCR	Median	OS	DE
2013 PLOS one [32]	China	ESCC	< = 60y 105 > 60y 144	M 136 F 113	249	I-IV	Over 60	Tissue	MISH	Normal	OS	DE
2014 MBR [23]	China	ESCC	Mean 61.4 (33–81)	M 115 F 79	194	I-IV	Median 31.3	Tissue blood	qRT-PCR	Mean	OS	Report
2015 AO [24]	Denmark	ESCC/EAC	32-86	M 140 F 55	195	I-IV	Median 24 ESCC	FFPE Tissue	Microarrays	Median	OS	Report
2015 IJCEM[33]	China	ESCC	< = 65y 21 > 65y 17	M 30 F 8	38	I-IV	Median 22	blood	qRT-PCR	Median	PFS/OS	DE
2015 IJO[34]	Japan	ESCC	< = 65y 37 > 65y 48	M 70 F 15	85	I-IV	Over 60	Tissue	qRT-PCR	Normal	OS	DE
2016 AJTR [19]	China	ESCC	< = 60y 68 > 60y 58	M 56 F 70	126	I-IV	Mean 19.9	blood	qRT-PCR	Median	OS	DE
2017 CEM[35]	China	ESCC	< = 65y 31 > = 65y 57	M 69 F 19	88	I-III	Over 20	Tissue	qRT-PCR	Mean	OS	Report

ESCC: esophageal squamous cell cancer; OS: overall survival; PFS: progression-free survival; DE: data extrapolated.

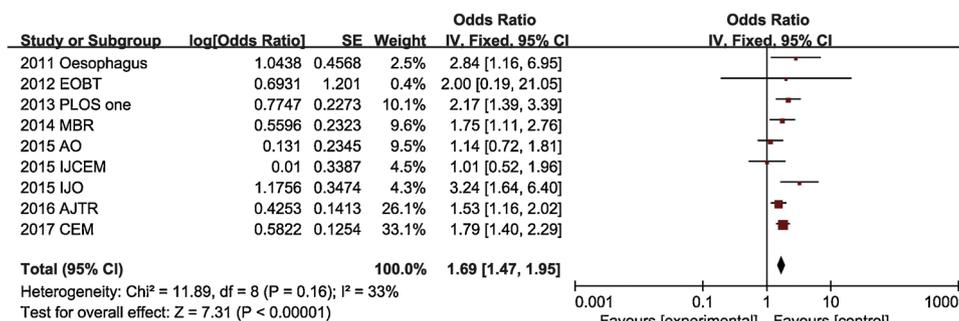


Fig. 5. Forest plot of the relationship between miR-375 expression and overall survival (OS) in patients with ESCC.

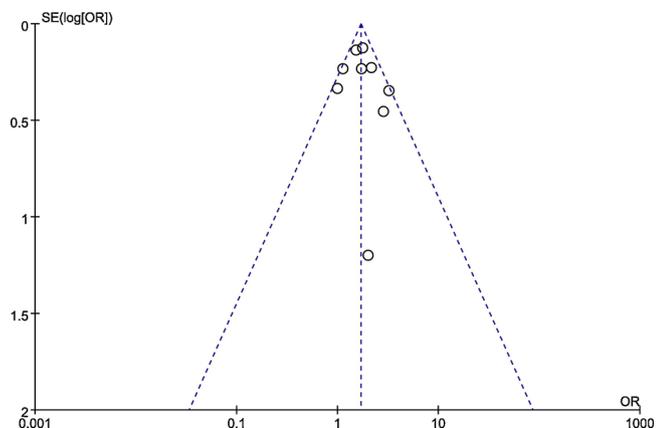


Fig. 6. Funnel plot of miR-375 expression and overall survival in patients with ESCC.

demonstrated a symmetric funnel-shape distribution (Fig. 6). Hence, there was no evidence for significant publication bias in the meta-analysis.

4. Discussion

ESCC is a highly lethal malignant tumor due to lack of effective treatment once the recurrence occurs after surgery or chemo-radiotherapy [13]. If the biological behavior of the tumor could be reliably predicted at the time of initial diagnosis, the prognosis of patients with esophageal cancer will be improved considerably. Therefore, understanding the molecular mechanisms of esophageal cancer development and identification of novel biomarkers is urgently needed. Growing evidence has demonstrated that the miRNAs were promising biomarkers for early cancer detection and prognosis prediction as well as therapy targets for molecular targeting treatment [14,15]. In the present study, 129 differentially expressed miRNAs were identified, and eight of them were associated with overall survival in patients with ESCC. We subsequently focused on the miR-375 and found it an independent prognostic factor for patients with ESCC. Moreover, we screened the candidate target genes of miR-375 and identified the key pathways and gene sets that altered between the patients with miR-375 low expression and high expression using bioinformatics methods. At the last part, we investigated the prognostic significance of the miR-375 using meta-analysis.

MiR-375 is located on human chromosome 2q35 and first identified as a pancreatic islet-specific miRNA regulating insulin secretion [16]. However, further study revealed that miR-375 was dysregulated in various tumor types, including breast cancer [17], head and neck cancer [18], esophageal cancer [19] and gastric carcinoma [20], implicating that miR-375 may play an important role in cancer progression. Actually, a number of retrospective studies have suggested that

miR-375 could potentially represent a tumor suppressor in patients with ESCC, with low expression of miR-375 associating with poorer survival [21–23]. However, contradicting results have been reported by Winther et al who found that high expression of miR-375 was not associated with enhanced survival in either ESCC or EAC [24]. These inconsistent results were due to the bias from the limited sample sizes, cut-off definitions and different origins including FFPE tumor specimens, frozen tumor tissues, serum and plasma. In order to reduce the bias, we downloaded the high-throughput data including the miR-seq and mRNA-seq data for the ESCC from TCGA to screen the miRNAs that were associated with the outcomes of the patients with ESCC. As a result, eight miRNAs were found to be correlated with the outcome of the patients with ESCC. Furthermore, multivariate analysis identified that miR-375 was an independent prognostic marker for patients with ESCC. Consistently, a meta-analysis only enrolled the patient with ESCC confirmed that low expressed miR-375 was associated with poorer survival.

In previous studies, miR-375 was clarified to play as a tumor suppressor via inhibiting cell proliferation of esophageal squamous cell carcinoma (ESCC), colony formation ability and metastasis in vitro and in vivo [25,26]. Additionally, miR-375 could directly down-regulated its target genes IGF1R, PDK1 and AEG-1 to inhibit the PI3K/Akt signaling pathway, which could promotes aerobic glycolysis by enhancing glucose uptake and the activity of glycolytic enzymes, thus suppressing growth of cancer cell [26–28]. In the present study, in order to gain a deep insight into the molecular functions of miR-375, we predicted the candidate target genes of miR-375 by taking the intersection between three online tools and the DEGs. Four candidate genes, including POU3F1, ARL4C, ISL2 and HNF1B, were identified as the target genes of miR-375 (Table 5). According to previous studies, expression of ARL4C induced by a combination of Wnt/ β -catenin and epidermal growth factor/Ras signaling may promote tumorigenesis of lung cancer and colorectal cancer [29], indicating that ARL4C may be a novel therapeutic target. Therefore, we speculated that miR-375 target ARL4C to suppress proliferation of ESCC cell, which needed further molecular investigations both in vitro and in vivo to verify these hypothesis.

On the other hand, we performed Gene Set Enrichment Analyses (GSEA) to identify the key pathways and enriched gene sets. Interestingly, the top three gene sets enriched in the high miR-375 expression was involved in RNA polymerase I transcriptional activity and RNA polymerase III transcriptional activity, which is essential for sustained protein synthesis and a fundamental determinant of the cell growth activity [30,31]. According to previous studies, inactivations of tumor suppressors in cancers deregulated the activity of RNA polymerases I and III and promoted cancer cell grow. In the present study, gene sets involved in the activity of RNA polymerases I and III were negatively with miR-375 expression, indicating that patients with high miR-375 expression in ESCC had a well prognosis due to down-regulated activity of RNA polymerase I and III.

In conclusion, we demonstrated miR-375 aberrant low expression

could be a biomarker for poor prognosis prediction in patients with ESCC. Further well-designed studies and deep investigation about the molecular mechanism of miR-375 in ESCC are required to validate the results of our bioinformatics analysis.

Conflict of interest

Authors declare that there is no conflict of interest.

Acknowledgment

None.

References

- W. Chen, R. Zheng, P.D. Baade, S. Zhang, H. Zeng, F. Bray, A. Jemal, X.Q. Yu, J. He, Cancer statistics in China, 2015, *CA Cancer J. Clin.* 66 (2016) 115–132.
- J.G. Chen, H.Z. Chen, J. Zhu, Y.L. Yang, Y.H. Zhang, P.X. Huang, Y.S. Chen, C.Y. Zhu, L.P. Yang, K. Shen, F.L. Qiang, G.R. Wang, Cancer survival in patients from a hospital-based cancer registry, *China, J. Cancer* 9 (2018) 851–860.
- V. Oppedijk, A. van der Gaast, J.J. van Lanschot, P. van Hagen, R. van Os, C.M. van Rij, M.J. van der Sangen, J.C. Beukema, H. Rutten, P.H. Spruit, J.G. Reinders, D.J. Richel, M.I. van Berge Henegouwen, M.C. Hulshof, Patterns of recurrence after surgery alone versus preoperative chemoradiotherapy and surgery in the CROSS trials, *J. Clin. Oncol.* 32 (2014) 385–391.
- D.P. Bartel, MicroRNAs: target recognition and regulatory functions, *Cell* 136 (2009) 215–233.
- M. Seven, O.F. Karatas, M.B. Duz, M. Ozen, The role of miRNAs in cancer: from pathogenesis to therapeutic implications, *Future Oncol.* 10 (2014) 1027–1048.
- J. Zhang, J. Zhang, W. Qiu, et al., MicroRNA-1231 exerts a tumor suppressor role through regulating the EGFR/PI3K/AKT axis in glioma[J], *J. Neurooncol.* 139 (3) (2018) 547–562.
- S.L. Romero-Cordoba, I. Salido-Guadarrama, M. Rodriguez-Dorantes, A. Hidalgo-Miranda, miRNA biogenesis: biological impact in the development of cancer, *Cancer Biol. Ther.* 15 (2014) 1444–1455.
- F.Y. Yu, C.Y. Zhou, Y.B. Liu, B. Wang, L. Mao, Y. Li, miR-483 is down-regulated in gastric cancer and suppresses cell proliferation, invasion and protein O-GlcNAcylation by targeting OGT, *Neoplasia* 65 (2018) 406–414.
- C. Luo, D. Yin, H. Zhan, U. Borjigin, C. Li, Z. Zhou, Z. Hu, P. Wang, Q. Sun, J. Fan, J. Zhou, X. Wang, S. Zhou, X. Huang, microRNA-501-3p suppresses metastasis and progression of hepatocellular carcinoma through targeting LIN7A, *Cell Death Dis.* 9 (2018) 535.
- U.R. Chandran, O.P. Medvedeva, M.M. Barmada, P.D. Blood, A. Chakka, S. Luthra, A. Ferreira, K.F. Wong, A.V. Lee, Z. Zhang, R. Budden, J.R. Scott, A. Berndt, J.M. Berg, R.S. Jacobson, TCGA expedition: a data acquisition and management system for TCGA data, *PLoS One* 11 (2016) e0165395.
- A. Subramanian, P. Tamayo, V.K. Mootha, S. Mukherjee, B.L. Ebert, M.A. Gillette, A. Paulovich, S.L. Pomeroy, T.R. Golub, E.S. Lander, J.P. Mesirov, Gene set enrichment analysis: a knowledge-based approach for interpreting genome-wide expression profiles, *Proc. Natl. Acad. Sci. U. S. A.* 102 (2005) 15545–15550.
- J.F. Tierney, L.A. Stewart, D. Gheris, S. Burdett, M.R. Sydes, Practical methods for incorporating summary time-to-event data into meta-analysis, *Trials* 8 (2007) 16.
- T.C. Kok, A. Van der Gaast, J. Dees, W.M. Eykenboom, H. Van Overhagen, G. Stoter, H.W. Tilanus, T.A. Splinter, Cisplatin and etoposide in oesophageal cancer: a phase II study. Rotterdam Oesophageal Tumour Study Group, *Br. J. Cancer* 74 (1996) 980–984.
- G. Bertoli, C. Cava, I. Castiglioni, MicroRNAs: new biomarkers for diagnosis, prognosis, therapy prediction and therapeutic tools for breast cancer, *Theranostics* 5 (2015) 1122–1143.
- G. Cheng, Circulating miRNAs: roles in cancer diagnosis, prognosis and therapy, *Adv. Drug Deliv. Rev.* 81 (2015) 75–93.
- N.N. Barouk, E. Van Obberghen, Function of microRNA-375 and microRNA-124a in pancreas and brain, *FEBS J.* 276 (2009) 6509–6521.
- T. Erbes, M. Hirschfeld, G. Rucker, M. Jaeger, J. Boas, S. Iborra, S. Mayer, G. Gitsch, E. Sticleker, Feasibility of urinary microRNA detection in breast cancer patients and its potential as an innovative non-invasive biomarker, *BMC Cancer* 15 (2015) 193.
- K. Hudcova, M. Raudenska, J. Gumulec, H. Binkova, Z. Horakova, R. Kostrica, P. Babula, V. Adam, M. Masarik, Expression profiles of miR-29c, miR-200b and miR-375 in tumour and tumour-adjacent tissues of head and neck cancers, *Tumour Biol.* 37 (2016) 12627–12633.
- H. Lv, Z. He, H. Wang, T. Du, Z. Pang, Differential expression of miR-21 and miR-75 in esophageal carcinoma patients and its clinical implication, *Am. J. Transl. Res.* 8 (2016) 3288–3298.
- S.W. Lee, K.C. Park, J.G. Kim, S.J. Moon, S.B. Kang, D.S. Lee, H.J. Sul, J.S. Ji, H.Y. Jeong, Dysregulation of MicroRNA-196b-5p and MicroRNA-375 in gastric cancer, *J. Gastric Cancer* 16 (2016) 221–229.
- J. Yi, L. Jin, J. Chen, B. Feng, Z. He, L. Chen, H. Song, MiR-375 suppresses invasion and metastasis by direct targeting of SHOX2 in esophageal squamous cell carcinoma, *Acta Biochim. Biophys. Sin. (Shanghai)* 49 (2017) 159–169.
- S. Komatsu, D. Ichikawa, H. Takeshita, H. Konishi, H. Nagata, S. Hirajima, T. Kawaguchi, T. Arita, A. Shiozaki, H. Fujiwara, K. Okamoto, E. Otsuji, Prognostic impact of circulating miR-21 and miR-375 in plasma of patients with esophageal squamous cell carcinoma, *Expert Opin. Biol. Ther.* 12 (Suppl 1) (2012) S53–59.
- C. Wu, M. Li, C. Hu, H. Duan, Clinical significance of serum miR-223, miR-25 and miR-375 in patients with esophageal squamous cell carcinoma, *Mol. Biol. Rep.* 41 (2014) 1257–1266.
- M. Winther, J. Alsner, T. Tramm, L. Baeksgaard, E. Holtved, M. Nordmark, Evaluation of miR-21 and miR-375 as prognostic biomarkers in esophageal cancer, *Acta Oncol. (Madr)* 54 (2015) 1582–1591.
- C. Hu, L. Lv, J. Peng, D. Liu, X. Wang, Y. Zhou, J. Huo, MicroRNA-375 suppresses esophageal cancer cell growth and invasion by repressing metadherin expression, *Oncol. Lett.* 13 (2017) 4769–4775.
- K.L. Kong, D.L. Kwong, T.H. Chan, S.Y. Law, L. Chen, Y. Li, Y.R. Qin, X.Y. Guan, MicroRNA-375 inhibits tumour growth and metastasis in oesophageal squamous cell carcinoma through repressing insulin-like growth factor 1 receptor, *Gut* 61 (2012) 33–42.
- N. Nohata, T. Hanazawa, N. Kikkawa, M. Mutallip, D. Sakurai, L. Fujimura, K. Kawakami, T. Chiyomaru, H. Yoshino, H. Enokida, M. Nakagawa, Y. Okamoto, N. Seki, Tumor suppressive microRNA-375 regulates oncogene AEG-1/MTDH in head and neck squamous cell carcinoma (HNSCC), *J. Hum. Genet.* 56 (2011) 595–601.
- X. Li, R. Lin, J. Li, Epigenetic silencing of microRNA-375 regulates PDK1 expression in esophageal cancer, *Dig. Dis. Sci.* 56 (2011) 2849–2856.
- S. Matsumoto, S. Fujii, A. Kikuchi, Arl4c is a key regulator of tubulogenesis and tumorigenesis as a target gene of Wnt-beta-catenin and growth factor-Ras signalling, *J. Biochem.* 161 (2017) 27–35.
- T.W. Turowski, D. Tollervey, Transcription by RNA polymerase III: insights into mechanism and regulation, *Biochem. Soc. Trans.* 44 (2016) 1367–1375.
- K.M. Hannan, E. Sanij, L.I. Rothblum, R.D. Hannan, R.B. Pearson, Dysregulation of RNA polymerase I transcription during disease, *Biochim. Biophys. Acta* 1829 (2013) 342–360.
- J. Li, X. Li, Y. Li, et al., Cell-specific detection of miR-375 downregulation for predicting the prognosis of esophageal squamous cell carcinoma by miRNA in situ hybridization[J], *PLoS One* 8 (1) (2013) e53582.
- B.X. Li, Q. Yu, Z.L. Shi, et al., Circulating microRNAs in esophageal squamous cell carcinoma: association with locoregional staging and survival[J], *Int. J. Clin. Exp. Med.* 8 (5) (2015) 7241–7250.
- Y. Isozaki, I. Hoshino, Y. Akutsu, et al., Usefulness of microRNA375 as a prognostic and therapeutic tool in esophageal squamous cell carcinoma[J], *Int. J. Oncol.* 46 (3) (2015) 1059–1066.
- Y. He, J. Jin, L. Wang, et al., Evaluation of miR-21 and miR-375 as prognostic biomarkers in esophageal cancer in high-risk areas in China[J], *Clin. Exp. Metastasis* 34 (1) (2017) 73–84.