Int J Clin Exp Med 2017;10(1):563-574
www.ijcem.com /ISSN:1940-5901/IJCEM0038185

Original Article

Appraising microRNA-99a as a novel prognostic biomarker across 21 human cancer types based on data from the cancer genome atlas

Wen-Jie Chen[1], Xia Yang[1], Zhi-Hua Ye[1], Gang Chen[1], Sheng-Hua Li[2]

Departments of [1]Pathology, [2]Urological Surgery, First Affiliated Hospital of Guangxi Medical University, 6 Shuangyong Road, Nanning 530021, Guangxi Zhuang Autonomous Region, P. R. China

Received August 19, 2016; Accepted October 15, 2016; Epub January 15, 2017; Published January 30, 2017

Abstract: Background: MicroRNA-99a has been believed to play a critical role in progression in various cancers; however, the prognostic and clinicopathological value of microRNA-99a in cancers remains unclarified. Methods and Results: We first evaluated the prognostic significance of microRNA-99a expression in 21 human cancer types in The Cancer Genome Atlas (TCGA). Patients with lower microRNA-99a levels had lower chances of overall survival in lung adenocarcinoma, esophageal cancer, cervical cancer, and head and neck squamous cell carcinoma, whereas they were more likely to survive urothelial bladder cancer and cutaneous melanoma. Subsequently, we included seven studies with sufficient data to further assesse microRNA-99a expression and its prognostic significance in human cancer types using meta-analysis. Low microRNA-99a level was closely linked with poor survival according to the published studies (HR=2.531, 95% CI: 1.920~3.337), but not in TCGA data (HR=1.042, 95% CI: 0.971-1.119). It clearly showed that down regulation of microRNA-99a was closely associated with shorter survival according to published studies and TCGA data (HR=1.101, 95% CIs 1.028~1.179). Finally, the bioinformatics analysis revealed that microRNA-99a might be involved in complex cellular pathways by Gene Ontology (GO) Biological Processes term, such as regulation of transcription. Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis indicated that glycosaminoglycan biosynthesis and mitogen-activated protein kinase (MAPK) pathway were the most significant pathways regulated by microRNA-99a. The Search Tool for the Retrieval of Interacting Genes/Proteins (STRING) also visualized the protein interaction and the related pathways in this study. Conclusions: These results indicate that microRNA-99a may be a reliable indicator for the progression and outcome in cancers patients.

Keywords: MicroRNA-99a, cancers, prognostic, TCGA

Introduction

As the second leading cause of death in the worldwide, cancer is a complex and heterogeneous disease for entirely different genetic reasons [1-3]. The breakthrough in microarray and next generation sequencing technologies has facilitated development of a catalogue of genomic changes. Identifying new tumor biomarkers is of great value for diagnosis, life quality improvement and increase of survival chance for patients with cancers. However, so far, the intrinsic mechanism of cancer has not been well elucidated; therefore, there is an urgent need to clarify the molecular biology of cancer and identifying cancer therapeutic targets clinical purposes.

MicroRNAs (miRNAs), 17-25 nucleotides in length, are a diverse group of endogenous small nucleotide RNA molecules. MicroRNAs, as post-transcriptional regulators, have been proved to be involved in silencing their mRNA target by binding to messenger RNA (mRNA) at the post transcriptional level [4]. MicroRNAs have played a vital roles in multiple developmental and physiological processes, such as hematopoiesis, differentiation and apoptosis [5, 6]. Recent studies have revealed that dysregulated microRNAs are able to function as tumor suppressor and oncogenic factorsin human cancer [7-9].

The microRNA-99a (miR-99a), belonging to the miR-99 family, islocated at the intron 13 of the

C21 or f34 gene at chromosome 21q. Compelling evidence suggested that miR-99a is implicated in carcinogenesis and cancer progression of various human malignancies. In addition, growing evidences revealed that miR-99a is frequently downregulated in human cancers, including oral cancer, cervical cancer and hepatocellular carcinoma [10-12]. However, to date, the role of miR-99a in patients with cancers has not been clarified yet. Further investigations into interactions between miR-99a and the patients with cancers are needed to provide profound insights into the role of miR-99a in cancers. Thus, we attempted to utilize the power of TCGA dataset and the results of individual studies to explore the prognostic efficiency of miR-99a in patients with cancers.

Materials and methods

TCGA cancer dataset

A literature research was performed to identify miR-99a expression profiling datasets comparing cancer and normal tissue. We obtained miR-99a expression data and corresponding clinical data for the cancer patients by using The Cancer Genome Atlas (TCGA) data portal [TCGA Data Portal. [https://tcga-data.nci.nih.gov/tcga/tcgaHome2.jsp]]. The TCGA data, including the miR-99a expression data and follow-up data of TCGA patients, are publicly available at the Data Coordinating Center (DCC). The Array tool was used to collect normalized miRNA expression data from the TCGA Data Portal [13].

Selection of studies

We conducted a systematic search in the electronic databases up to August 1st 2016, including PubMed and Web of Science, using the MESH search headings: (miR-99a OR miRNA-99a OR microRNA99a OR miR99a OR miRNA99a OR microRNA99a OR miR 99a OR miRNA 99 OR microRNA 99a OR miR-99a-5p OR miRNA-99a-5p OR microRNA-99a-5p) AND (cancer OR carcinoma OR adenocarcinoma OR sarcoma OR tumor OR neoplas* OR malignan*).

Inclusion criteria and exclusion criteria

All studies for analysis met following criteria: (i), they investigated the association between miR-99a expression levels and cancer prognosis; (ii), the expression of miR-99a was evaluated by qRT-PCR, miRNA microarray or sequencing methods. (iii), the studies reported the necessary data to calculate or extrapolate from the published results. Studies were considered ineligible for the meta-analysis if: (i) studies did not meet the inclusion criteria, (ii) studies did not provide sufficient data for calculation, (iii) studies used cell lines sample.

Data extraction

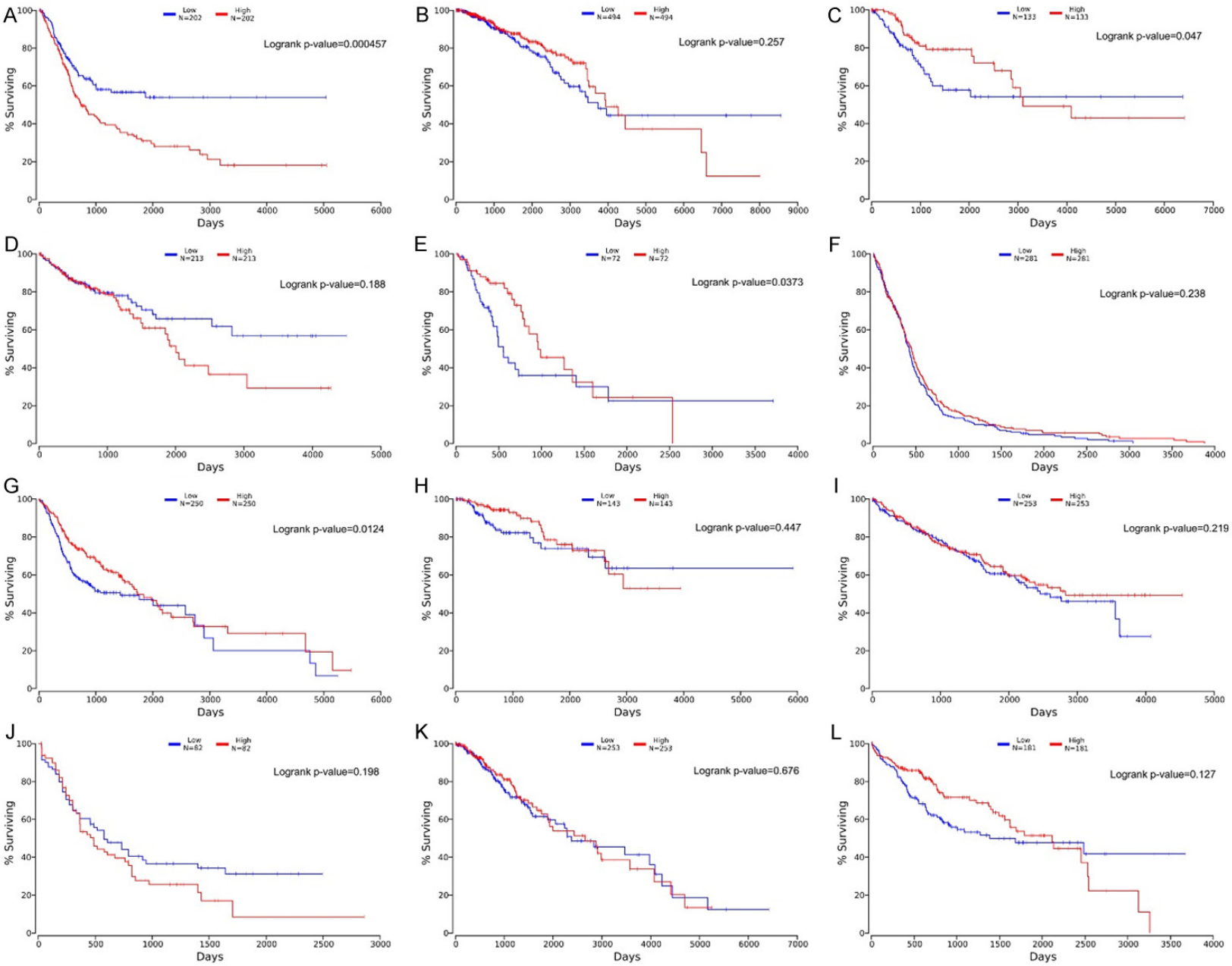
Data extraction was repeated by two independent readers (WJ C and ZH Y). Extracted data, which included first author's name, publication year, tumor type, and number of patients, survival and HR with confidence intervals (CIs), were recorded independently by both investigators. Disagreements were resolved by consensus necessary.

Target gene search for miR-99a and enrichment analysis

The putative target genes of miR-99a were predicted by twelve programs: miRWalk, Microt4, miRanda, mirbridge, miRDB, miRMap, miRNAMap, Pictar2, PITA, RNA22, RNAhybrid and Targetscan. The target genes which existed in at least four datasets were selected. The enrichment analysis of the potential targeted genes, including Gene Ontology (GO) Biological Processes term and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway, were conducted by using DAVID online analysis (https://david.ncifcrf.gov/) [14]. In regulatory network analysis, we predicted the association between miR-99a and the target gene by using the STRING database (the Search Tool for the Retrieval of Interacting Genes/Proteins) [15].

Statistical analysis

For survival analysis, we analyzed the correlation between overall survival and the miR-99a by the Kaplan-Meier method, while the log-rank test was used to compare survival curves by. Using the Cox regression models, we calculated hazard ratios for the miR-99a relative prognostic value, from survival analysis. The intensity of association between miR-99a level and survival was described by HR. An HR greater than 1 suggested worse prognosis in patients with low miR-99a level. Otherwise, we used the soft-


A
Low N=202
High N=202
Logrank p-value=0.000457
B
Low N=494
High N=494
Logrank p-value=0.257
C
Low N=133
High N=133
Logrank p-value=0.047
D
Low N=213
High N=213
Logrank p-value=0.188
E
Low N=72
High N=72
Logrank p-value=0.0373
F
Low N=281
High N=281
Logrank p-value=0.238
G
Low N=250
High N=250
Logrank p-value=0.0124
H
Low N=143
High N=143
Logrank p-value=0.447
I
Low N=253
High N=253
Logrank p-value=0.219
J
Low N=82
High N=82
Logrank p-value=0.198
K
Low N=253
High N=253
Logrank p-value=0.676
L
Low N=181
High N=181
Logrank p-value=0.127
% Surviving
Days

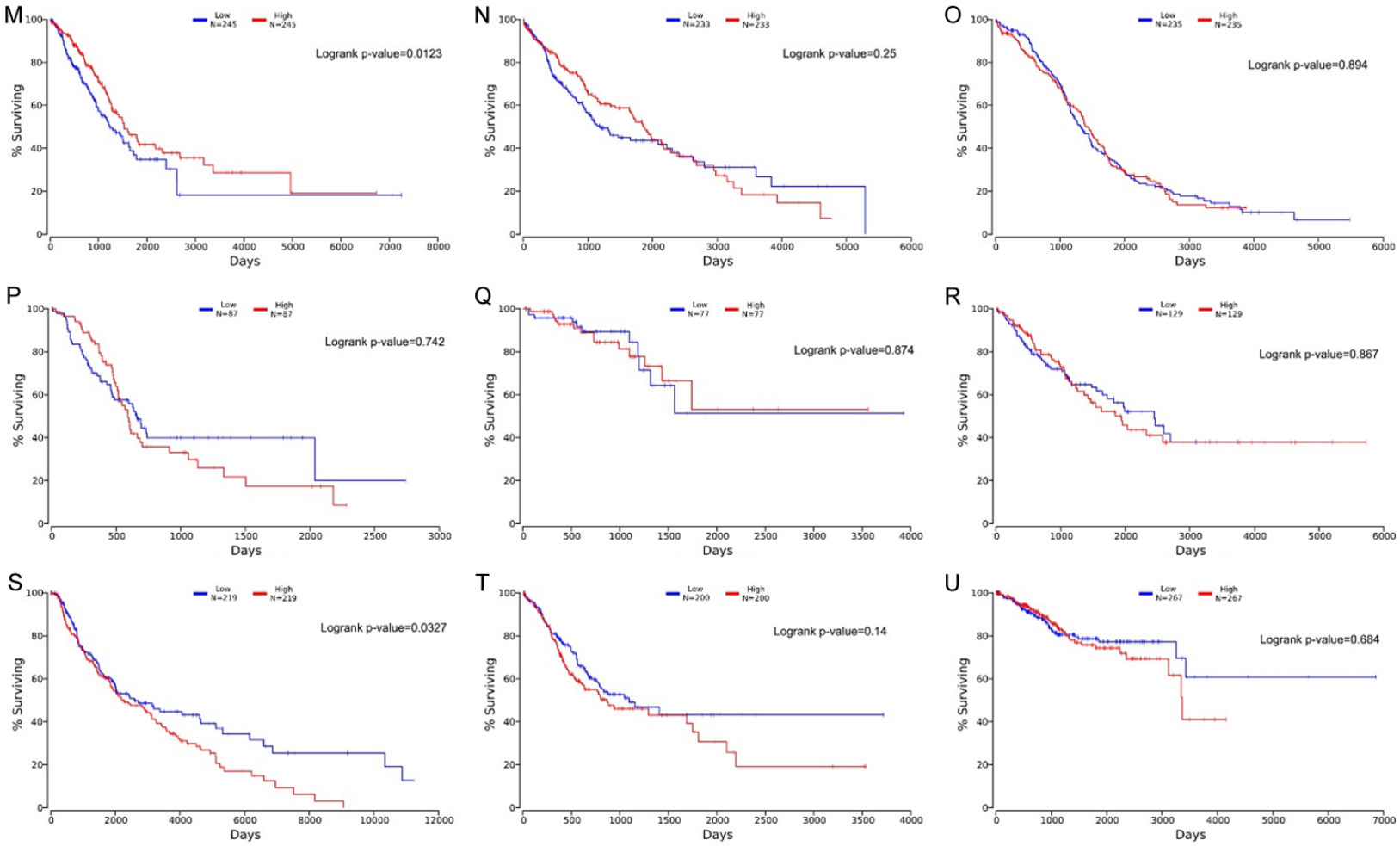


Figure 1. Kaplan-Meier survival curves for miR-99a expression levels on overall survival based on TCGA according to cancers subtypes. A. Urothelial bladder cancer (BLCA). B. Breast cancer (BRCA). C. Cervical cancer (CESC). D. Colon adenocarcinoma (COAD). E. Esophageal cancer (ESCA). F. Glioblastoma multiforme (GBM). G. Head and neck squamous cell carcinoma (HNSC). H. Clear cell kidney carcinoma (KIRC). I. Papillary kidney carcinoma (KIRP). J. Acute Myeloid Leukemia (LAML). K. Lower Grade Glioma (LGG). L. Liver hepatocellular carcinoma (LIHC). M. Lung adenocarcinoma (LUAD). N. Lung squamous cell carcinoma (LUSC). O. Ovarian serous cystadenocarcinoma (OV). P. Pancreatic ductal adenocarcinoma (PAAD). Q. Rectal adenocarcinoma (READ). R. Sarcoma (SARC). S. Cutaneous melanoma (SKCM). T. Stomach adenocarcinoma (STAD). U. Uterine corpus endometrial carcinoma (UCEC).

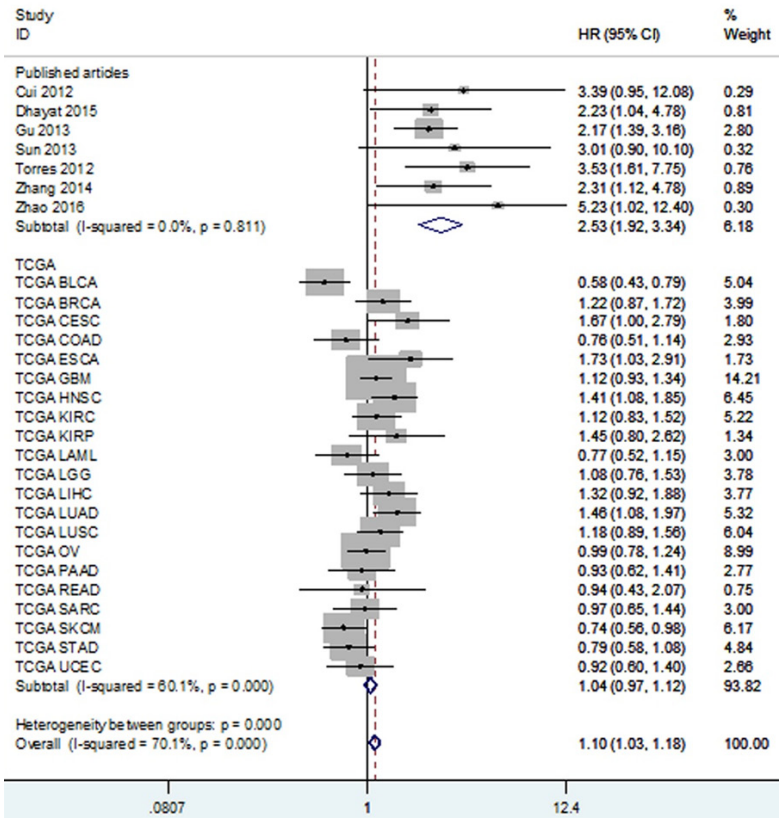


Figure 2. A Forest Plot of the pooled HR form fixed-effect OS.

ware Engauge Digitizer version 4.1 (http://digitizer.sourceforge.net/) estimated HR and 95% CIs from Kaplan-Meier survival curves by the method described previously [16], when HR and 95% CIs could not be specified within the studies. Next, Chi-squared test (Chi^2) and inconsistency (I^2) was used to test statistical heterogeneity [17, 18]. The DerSimonian and Laird method (random effects model)were employed to pool and HRs with 95% CIs by the random effects model (I^2 more than 50.0%) [19]. A two-side *P*-value <0.05 wasconsidered to be statistically significant. STATA version 12.0 software was used for the study.

Results

TCGA data analysis

Selection of the TCGA datasets: A total of twenty-one miR-99a expression profiles with sufficient data for the different cancers were identified in the TCGA datasets. Twenty-one cancers were reported in the 21 miRNA expression profiling datasets with 8498 patients. The clinical data with survival time and status for those patients were available in TCGA datasets. For survival analysis, we divided the patients into the low miR-99a level group and high miR-99a level group respectively for each cancer type by 50 percent.

MiR-99a expression and overall survival in cancers: We tried to evaluate the prognostic power of miR-99a using the TCGA dataset of 21 cancers. Consistent with the result reported by the previous study [20], decreased expression of miR-99a were associated with a poor prognosis in lung adenocarcinoma. Additionally, we discovered significant correlations between low expression of miR-99a and poor survival in cervical cancer, esophageal cancer and head and neck squamous cell carcinoma (HR=1.671, 95% CIs 1.001~2.790; HR=1.729, 95% CIs 1.026~2.913; HR=1.410, 95% CIs 1.076~1.848, respectively) (**Figure 2**). On the contrary, a higher miR-99a expression was found to be predictive of shorter OS in urothelial bladder cancer (HR=0.582; 95% CIs 0.429~0.791), as well as in cutaneous melanoma (HR=0.741; 95% CIs 0.562~0.977). MiR-99a expression did not show any significant relationship with survival in other cancers. Kaplan-Meier curves for low-expressed and the high-expressed miR-99a groups was shown in **Figure 1** based on TCGA cohort.

Meta-analysis

Characteristics of included studies: Three hundred and fifty articles were retrieved in the electronic literatures. Seven studies between 2012 and 2016 met our inclusion criteria, including 647 patients with cancers [10, 20-25]. Patient tissues were the most complete cancers samples used to detect miR-99a, whereas in one studies the authors used plasma specimens to assess miR-99a [22]. The main characteristics of the included studies are outlined in **Table 1**.

Table 1. Characteristics of articles included in the meta-analysis

Author Year	Cancer Type	Number	OS
			HR (95% CIs)
Gu 2013	Lung Adenocarcinoma	96	2.170 (1.390, 3.160)
Torres 2012	Endometrioid endometrial carcinoma	77	3.533 (1.608, 7.751)
Zhao 2016	Osteosarcoma	130	5.232 (1.022, 12.398)
Sun 2013	Esophageal squamous cell carcinoma	61	3.012 (0.898, 10.101)
Dhayat 2015	Pancreatic ductal adenocarcinoma	91	2.229 (1.040, 4.778)
Cui 2012	Renal cell carcinoma	40	3.393 (0.953, 12.080)
Zhang 2014	Hepatocellular carcinoma	152	2.309 (1.115, 4.785)
TCGA BLCA	Urothelial bladder cancer	404	0.582 (0.429, 0.791)
TCGA BRCA	Breast cancer	988	1.220 (0.865, 1.721)
TCGA CESC	Cervical cancer	266	1.671 (1.001, 2.790)
TCGA COAD	Colon adenocarcinoma	426	0.764 (0.512, 1.142)
TCGA ESCA	Esophageal cancer	144	1.729 (1.026, 2.913)
TCGA GBM	Glioblastoma multiforme	562	1.116 (0.930, 1.339)
TCGA HNSC	Head and neck squamous cell carcinoma	500	1.410 (1.076, 1.848)
TCGA KIRC	Clear cell kidney carcinoma	506	1.124 (0.832, 1.518)
TCGA KIRP	Papillary kidney carcinoma	286	1.449 (0.800, 2.624)
TCGA LAML	Acute Myeloid Leukemia	164	0.773 (0.520, 1.149)
TCGA LGG	Lower Grade Glioma	506	1.078 (0.757, 1.535)
TCGA LIHC	Liver hepatocellular carcinoma	362	1.316 (0.924, 1.875)
TCGA LUAD	Lung adenocarcinoma	490	1.460 (1.084, 1.967)
TCGA LUSC	Lung squamous cell carcinoma	466	1.178 (0.891, 1.558)
TCGA OV	Ovarian serous cystadenocarcinoma	470	0.985 (0.783, 1.238)
TCGA PAAD	Pancreatic ductal adenocarcinoma	174	0.933 (0.618, 1.410)
TCGA READ	Rectal adenocarcinoma	154	0.938 (0.425, 2.071)
TCGA SARC	Sarcoma	258	0.967 (0.650, 1.437)
TCGA SKCM	Cutaneous melanoma	438	0.741 (0.562, 0.977)
TCGA STAD	Stomach adenocarcinoma	400	0.791 (0.579, 1.081)
TCGA UCEC	Uterine corpus endometrial carcinoma	534	0.916 (0.601, 1.396)

Association between miR-99a and survival in 21 types of cancers: To gain further insights into the prognostic value of miR-99a expression in cancers, we firstly analyzed the association of miR-99a expression and overall survival in the included studies. The down regulation of miR-99a conferred the poor OS for cancers patients in random model (pooled HR=2.531, 95% CIs 1.920~3.337, **Table 2**) with no obvious heterogeneity observed (I^2=0.00%, P=0.811). Conversely, the analysis did not exhibit a prognostic effect of decreased miR-99a expression for OS for the TCGA dataset (pooled HR=1.045, 95% CIs 0.928~1.176) using the fixed-effects model. Subsequently, we conducted a meta-analysis of the prognostic value of miR-99a expression in cancers patients with all the included data. It was also clearly showed that down regulation of mir-99a is significantly associated with the shorter survival (pooled HR=1.101, 95% CIs 1.028~1.179; **Figure 2**). To further clarify these results, we conducted the subgroup analysis miR-99a by cancer type. Low level of miR-99a expression significantly revealed a predictive value for survival in lung adenocarcinoma, esophageal cancer and liver hepatocellular carcinoma (HR=1.674, 95% CIs 1.315~2.130; HR=1.886, 95% CIs 1.168~3.046; HR=1.465, 95% CIs 1.066, 2.014, respectively), but not in the other cancers (**Table 2**).

Target gene search for miR-99a and enrichment analysis

We identified a total of 28277 genes as potential targets of miR-99a in twelve up-to-date

Table 2. Summarized HRs of overall analyses for OS

Stratified analysis	Study (N)	HR (95% CIs)		Heterogeneity	
		Fixed	Random	I^2	p
ALL	28	1.101 (1.028, 1.179)	1.188 (1.036, 1.363)	70.10%	<0.001
Data source					
Published articles	7	2.531 (1.920, 3.337)	2.531 (1.920, 3.337)	0.00%	0.811
TCGA	21	1.042 (0.971, 1.119)	1.045 (0.928, 1.176)	60.10%	<0.001
Cancer type					
KIRC	2	1.192 (0.890, 1.597)	1.632 (0.586, 4.545)	63.70%	0.097
PAAD	2	1.136 (0.791, 1.633)	1.356 (0.583, 3.157)	74.20%	0.049
LUAD	2	1.674 (1.315, 2.130)	1.734 (1.180, 2.548)	57.30%	0.126
ESCA	2	1.886 (1.168, 3.046)	1.886 (1.168, 3.046)	0.00%	0.409
UCEC	2	1.238 (0.854, 1.795)	1.724 (0.460, 6.458)	88.60%	0.003
LIHC	2	1.465 (1.066, 2.014)	1.587 (0.944, 2.668)	46.00%	0.174
SARC	2	1.129 (0.774, 1.648)	2.019 (0.391, 10.149)	84.30%	0.011

Table 3. GO processes most strongly enriched by miR-99a targets

Term	Count	*P* Value	FDR
Biological processes			
GO:0045449~regulation of transcription	91	0.000036700	0.063258
GO:0006350~transcription	77	0.000042900	0.073866
GO:0045892~negative regulation of transcription, DNA-dependent	21	0.000270000	0.464742
GO:0051253~negative regulation of RNA metabolic process	21	0.000338000	0.580491
GO:0048259~regulation of receptor-mediated endocytosis	5	0.000426000	0.730803
GO:0000122~negative regulation of transcription from RNA polymerase II promoter	17	0.000526000	0.902977
GO:0006342~chromatin silencing	5	0.000690000	1.181657
GO:0048260~positive regulation of receptor-mediated endocytosis	4	0.000955000	1.63279
GO:0016481~negative regulation of transcription	23	0.001170543	1.997349
GO:0045814~negative regulation of gene expression, epigenetic	5	0.001276675	2.176577
Cellular components			
GO:0016585~chromatin remodeling complex	8	0.001151769	1.538008
GO:0012505~endomembrane system	31	0.003675115	4.831318
GO:0005624~membrane fraction	30	0.010571242	13.31888
GO:0044459~plasma membrane part	66	0.015180073	18.59463
GO:0005626~insoluble fraction	30	0.017176609	20.78645
GO:0005794~Golgi apparatus	30	0.026628927	30.44128
GO:0005654~nucleoplasm	30	0.030472127	34.04563
GO:0005731~nucleolus organizer region	2	0.04516912	46.29408
GO:0030874~nucleolar chromatin	2	0.04516912	46.29408
GO:0034702~ion channel complex	10	0.046092468	46.98839
Molecular function			
GO:0030528~transcription regulator activity	55	0.00122228	1.767194
GO:0003677~DNA binding	77	0.001549107	2.234773
GO:0003700~transcription factor activity	37	0.005016706	7.069688
GO:0008146~sulfotransferase activity	6	0.005826423	8.166143
GO:0016782~transferase activity, transferring sulfur-containing groups	6	0.011794447	15.88353
GO:0016791~phosphatase activity	13	0.015480462	20.34358
GO:0004721~phosphoprotein phosphatase activity	10	0.01661147	21.66729
GO:0043565~sequence-specific DNA binding	24	0.01793241	23.18734
GO:0046332~SMAD binding	5	0.022964355	28.72971
GO:0043167~ion binding	118	0.023902722	29.72113

prediction algorithms. Then, we observed an overlap of 828 targeted genes enriched at least in four data sets. The vital role miR-99 as functionally cooperative target and biologically relevant genes in signaling and biological pathways was detected by geneset enrichment analysis. A total of 179 of the biological functions were confirmed by GO analysis. The top ten GO processes which were most significantly enriched with respect to the miR-99a candidates are presented in **Table 3**. The top enriched biological process was regulation of transcription (**Figure 3A-C**). A total of 212 KEGG pathways were enriched by miR-99a signature, of which nine pathways were significant with <0.005. The top nine functional enrichment of target genes for miR-99a signature were summarized in the **Table 4**. The potentially important functional role of predicted miR-99a targets involved in the critical pathways were revealed by this analysis such as: glycosaminoglycan biosynthesis, MAPK signaling pathway and signaling pathways regulating pluripotency of stem cells (**Figure 3D**). Altogether, the results of the overlapping gene suggested that aberrant expression of miR-99a might be involved in the critical pathways implicated in cancer progression. We used STRING to visualize the protein interaction. **Figure 4** showed the network with methods of target gene.

Discussion

In this study, we employed an integrated analysis approach to analyze the prognostic value of miR-99a across 21 human cancer types derived from TCGA datasets. The downregulated miR-99a were significantly associated with poorer survival in lung adenocarcinoma, cervical cancer, esophageal cancer and head and neck squamous cell carcinoma, which was consistent with the results of the previous study [24, 26, 27]. Nonetheless, the significant correlation was also found between low level of miR-99a expression and better outcome in urothelial bladder cancer and cutaneous melanoma. Interestingly, we further confirmed that lower levels of miR-99a expression significantly reduced overall survival through TCGA data and the published studies. In the contrary to our result, Zhou et al. analyzed the miR-99a expression profile of 84 paired bladder cancer through TCGA data and identified the prognostic value of miR-99a that predicted patient survival [28]. Moreover, with data identified from TCGA dataset, Xu et al. also found the low miR-99a level was associated with progression and prognosis of muscle-invasive bladder cancer patients [29]. The major reason for this disparity is that the TCGA data for bladder cancer has been updated with the larger cohort, compared with the two previous studies published in 2015.

Consequently, we initially conducted a meta-analysis based on TCGA dataset and the published studies to evaluate the prognostic value of miR-99a expression in cancers. These result highlighted the potential of miR-99a to facilitate clinical prognosis prediction for cancer patients. However, there are some limitations in our study. First, different technological platforms were used to detect miR-99a expression profiling datasets. Second, included studies were retrospective validation with small sample sizes. Third, partially due to the limitation of data, the multivariate Cox regression analysis was not used for TCGA dataset, which could better present the influence of multiple factors on outcome. Fourth, only normal tissue and tumor were used for comparison in our analysis.

The aberrant expression of miR-99a in cancers prompted us to explore whether miR-99a functions as a tumor suppressor. Notably, miR-99ahas been proved to be involved in the inhibition ofG1 cell cycle arrest by suppressing mTOR in several cancers [27, 30, 31], Previous evidence hypothesized that miR-99a regulated downregulation of IGF-1R (insulin-like growth factor 1 receptor), which played a crucial role in promoting cell proliferation and metastasis [32]. Moreover, miR-99a acted as suppressor on cancer cell proliferation, migration and invasion by decreasing MTMR3 protein (Myotubularin-related protein 3) in oral cancer [11]. In the light of previous studies, miR-99a was reported to directly or indirectly modulate FGFR3, PSA and AGO1 [10, 33, 34].

To date, many studies have experimentally validated a number of targets for miRNA-99a. Due tonumerous potential gene targets for the single microRNA, the pathway enrichment and network analysis were necessary for the overview of the biological processes and protein interaction. With the help of analysis of DAVID database, we found that miR-99a regulates many protein-coding genes involved in negative regulation of RNA metabolic process, regula-

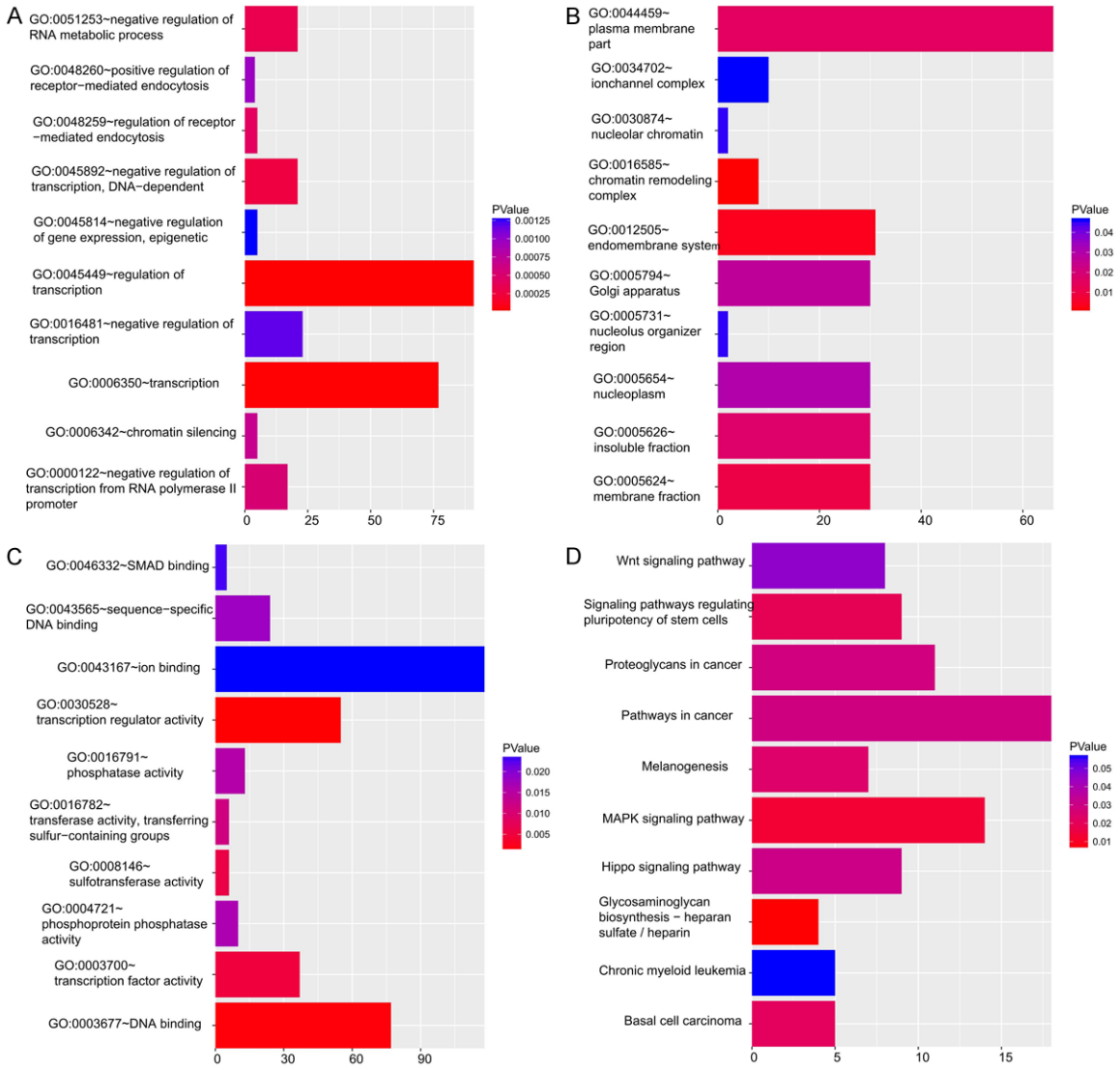


Figure 3. GO enrichment analysis (A-C) and KEGG pathway analysis (D). (A) Biological processes. (B) Cellular components. (C) Molecular function. (D) KEGG pathway.

tion of transcription and negative regulation of transcription. Of particular interest is the fact that miR-99a regulated gene expression profile showed the involvement of miR-99a in glycosaminoglycan biosynthesis-heparan sulfate (HS)/heparinpathway by pathway enrichment analysis. Notably, substantial evidence showed the expression of heparan sulfate proteoglycan was markedly altered in malignant transformation and tumor progression, by regulating the interactions between cells and signaling molecules [35]. The change of HS fine structure by key enzymes involved in HS biosynthesis and catabolism affected several downstream cellular processes of cancer progression. MiR-99a was known to be involved in MAPK pathway according to the KEGG analysis, which was in agreement with previous studies [36]. Previous studies have reported that miR-99a inhibits protein synthesis of tribbles pseudokinase 2 (TRIB2), which acts as a possible controller in the activation of MAPK and MAPK signaling [37]. Aberrant activation of the MAPK pathway may inhibit tumor-induced inflammation and angiogenesis, leading to cancer cells growth and metastasis in HNSCC [26].

In addition, the STRING analysis revealed three most frequent candidate genes that were regulated by miR-99a (forkhead box 03 FOXO3; protein phosphatase 3 catalytic subunit alpha, PPP3CA and mTOR). FOXO3 was known as a tumor suppressorgene in cancer development

Table 4. KEGG pathways most strongly enriched by miR-99a targets

KEGG Pathways	Count	*P* Value	Corrected *P*-Value
Glycosaminoglycan biosynthesis-heparan sulfate/heparin	4	0.006079	0.805532461
MAPK signaling pathway	14	0.013273	0.805532461
Signaling pathways regulating pluripotency of stem cells	9	0.019726	0.805532461
Basal cell carcinoma	5	0.021414	0.805532461
Melanogenesis	7	0.024068	0.805532461
Proteoglycans in cancer	11	0.028391	0.805532461
Pathways in cancer	18	0.029635	0.805532461
Hippo signaling pathway	9	0.030397	0.805532461
Wnt signaling pathway	8	0.045737	0.948050095

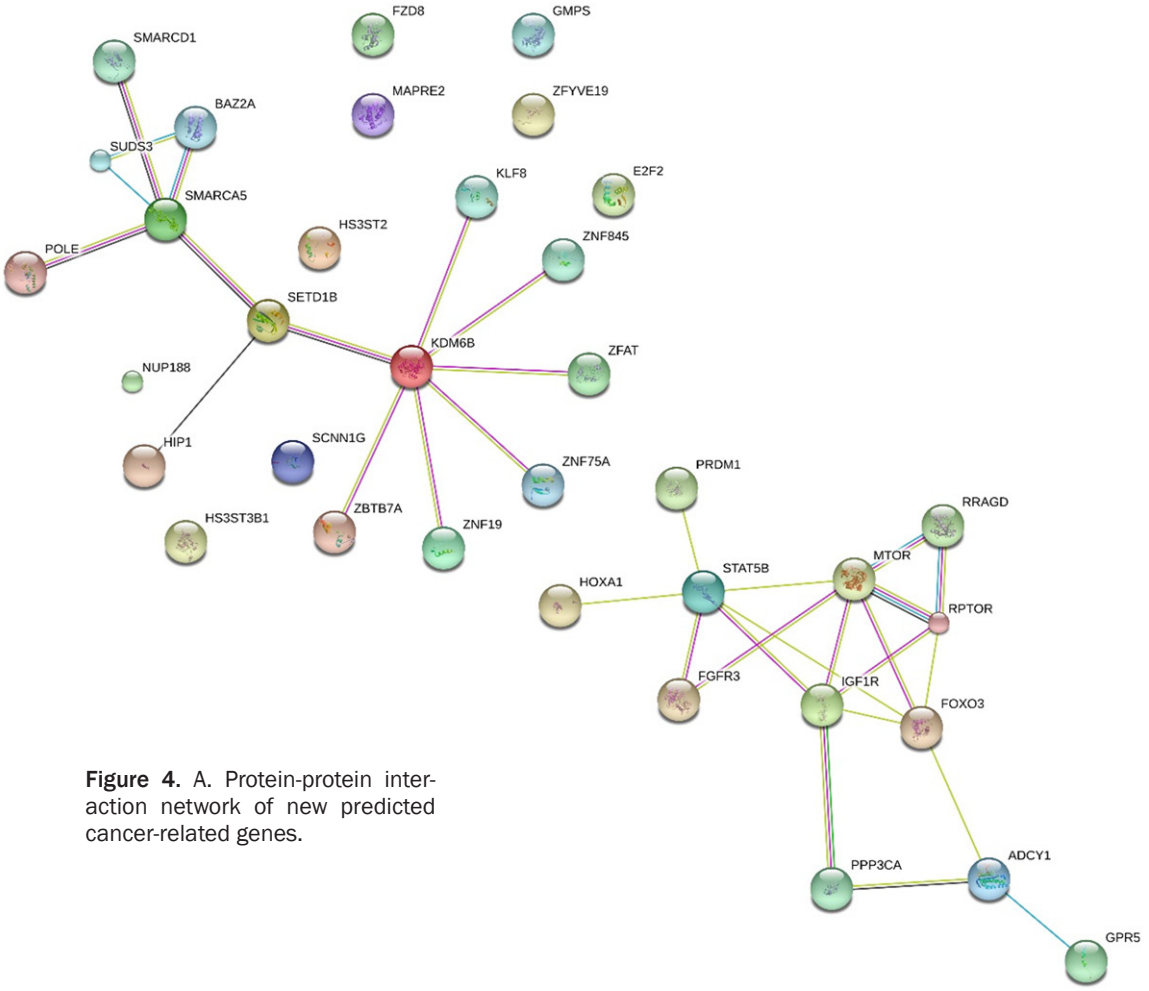


Figure 4. A. Protein-protein interaction network of new predicted cancer-related genes.

by increasing AKT activity or inactivating Phosphatase and tensin homolog (PTEN) [38, 39]. While PPP3CA was also discovered to be regulated by IGF-1R, DUSP4, TRIB2 and ADCY1, there is no other studies exploring the association between PPP3CA and miR-99a. Since certain false negatives and false positives do exit in the target prediction algorithms, further investigation is needed to elucidate the regulatory relationship between miR-99a and the vital pathway, which helps understand the role of miR-99a in cancers, and the patient outcome management.

Conclusion

This study suggests that miR-99a might potentially be a vital suppressor for many human can-

cer types, and highlights the prognostic value of miR-99a expression in cancer outcomes.

Acknowledgements

The study was supported partly by the Promoting Project of Basic Capacity for University Young and Middle-aged Teachers in Guangxi (KY2016LX034). The authors thank The Cancer Genome Atlas (TCGA) for the publicly available data.

Disclosure of conflict of interest

None.

Address correspondence to: Dr. Sheng-Hua Li, Department of Urological Surgery, First Affiliated Hospital of Guangxi Medical University, 6 Shuangyong Road, Nanning 530021, Guangxi Zhuang Autonomous Region, P. R. China. E-mail: 43538602@qq.com

References

[1] Burrell RA, McGranahan N, Bartek J and Swanton C. The causes and consequences of genetic heterogeneity in cancer evolution. Nature 2013; 501: 338-345.

[2] McGranahan N and Swanton C. Biological and therapeutic impact of intratumor heterogeneity in cancer evolution. Cancer Cell 2015; 27: 15-26.

[3] Siegel RL, Miller KD and Jemal A. Cancer statistics, 2016. CA Cancer J Clin 2016; 66: 7-30.

[4] Croce CM. Causes and consequences of microRNA dysregulation in cancer. Nat Rev Genet 2009; 10: 704-714.

[5] Esquela-Kerscher A and Slack FJ. Oncomirs-microRNAs with a role in cancer. Nat Rev Cancer 2006; 6: 259-269.

[6] Liu X, Chen X, Yu X, Tao Y, Bode AM, Dong Z and Cao Y. Regulation of microRNAs by epigenetics and their interplay involved in cancer. J Exp Clin Cancer Res 2013; 32: 96.

[7] Brunet Vega A, Pericay C, Moya I, Ferrer A, Dotor E, Pisa A, Casalots A, Serra-Aracil X, Oliva JC, Ruiz A and Saigi E. microRNA expression profile in stage III colorectal cancer: circulating miR-18a and miR-29a as promising biomarkers. Oncol Rep 2013; 30: 320-326.

[8] Matamala N, Vargas MT, Gonzalez-Campora R, Minambres R, Arias JI, Menendez P, Andres-Leon E, Gomez-Lopez G, Yanowsky K, Calvete-Candenas J, Inglada-Perez L, Martinez-Delgado B and Benitez J. Tumor microRNA expression profiling identifies circulating microRNAs for early breast cancer detection. Clin Chem 2015; 61: 1098-1106.

[9] Kang HS, Kim J, Jang SG, Kwon SY, Park YS, Green JE, Kim HK and Ro J. MicroRNA signature for HER2-positive breast and gastric cancer. Anticancer Res 2014; 34: 3807-3810.

[10] Zhang J, Jin H, Liu H, Lv S, Wang B, Wang R, Liu H, Ding M, Yang Y, Li L, Zhang J, Fu S, Xie D, Wu M, Zhou W and Qian Q. MiRNA-99a directly regulates AGO2 through translational repression in hepatocellular carcinoma. Oncogenesis 2014; 3: e97.

[11] Kuo YZ, Tai YH, Lo HI, Chen YL, Cheng HC, Fang WY, Lin SH, Yang CL, Tsai ST and Wu LW. MiR-99a exerts anti-metastasis through inhibiting myotubularin-related protein 3 expression in oral cancer. Oral Dis 2014; 20: e65-75.

[12] Wang L, Chang L, Li Z, Gao Q, Cai D, Tian Y, Zeng L and Li M. miR-99a and -99b inhibit cervical cancer cell proliferation and invasion by targeting mTOR signaling pathway. Med Oncol 2014; 31: 934.

[13] Yanaihara N, Caplen N, Bowman E, Seike M, Kumamoto K, Yi M, Stephens RM, Okamoto A, Yokota J, Tanaka T, Calin GA, Liu CG, Croce CM and Harris CC. Unique microRNA molecular profiles in lung cancer diagnosis and prognosis. Cancer Cell 2006; 9: 189-198.

[14] Huang da W, Sherman BT and Lempicki RA. Systematic and integrative analysis of large gene lists using DAVID bioinformatics resources. Nat Protoc 2009; 4: 44-57.

[15] Szklarczyk D, Franceschini A, Wyder S, Forslund K, Heller D, Huerta-Cepas J, Simonovic M, Roth A, Santos A, Tsafou KP, Kuhn M, Bork P, Jensen LJ and von Mering C. STRING v10: protein-protein interaction networks, integrated over the tree of life. Nucleic Acids Res 2015; 43: D447-452.

[16] Parmar MK, Torri V and Stewart L. Extracting summary statistics to perform meta-analyses of the published literature for survival endpoints. Stat Med 1998; 17: 2815-2834.

[17] Lau J, Ioannidis JP and Schmid CH. Quantitative synthesis in systematic reviews. Ann Intern Med 1997; 127: 820-826.

[18] Higgins JP and Thompson SG. Quantifying heterogeneity in a meta-analysis. Stat Med 2002; 21: 1539-1558.

[19] DerSimonian R and Laird N. Meta-analysis in clinical trials revisited. Contemp Clin Trials 2015; 45: 139-145.

[20] Gu W, Fang S, Gao L, Tan Y and Yang Z. Clinic significance of microRNA-99a expression in human lung adenocarcinoma. J Surg Oncol 2013; 108: 248-255.

[21] Cui L, Zhou H, Zhao H, Zhou Y, Xu R, Xu X, Zheng L, Xue Z, Xia W, Zhang B, Ding T, Cao Y, Tian Z, Shi Q and He X. MicroRNA-99a induces G1-phase cell cycle arrest and suppresses tumorigenicity in renal cell carcinoma. BMC Cancer 2012; 12: 546.

[22] Torres A, Torres K, Pesci A, Ceccaroni M, Paszkowski T, Cassandrini P, Zamboni G and Maciejewski R. Deregulation of miR-100, miR-99a and miR-199b in tissues and plasma coexists with increased expression of mTOR kinase in endometrioid endometrial carcinoma. BMC Cancer 2012; 12: 369.
[23] Dhayat SA, Abdeen B, Köhler G, Senninger N, Haier J and Mardin WA. MicroRNA-100 and microRNA-21 as markers of survival and chemotherapy response in pancreatic ductal adenocarcinoma UICC stage II. Clin Epigenetics 2015; 7: 132.
[24] Sun J, Chen Z, Tan X, Zhou F, Tan F, Gao Y, Sun N, Xu X, Shao K and He J. MicroRNA-99a/100 promotes apoptosis by targeting mTOR in human esophageal squamous cell carcinoma. Med Oncol 2013; 30: 411.
[25] Zhao J, Chen F, Zhou Q, Pan W, Wang X, Xu J, Ni L and Yang H. Aberrant expression of microRNA-99a and its target gene mTOR associated with malignant progression and poor prognosis in patients with osteosarcoma. Onco Targets Ther 2016; 1589.
[26] Hou B, Ishinaga H, Midorikawa K, Shah SA, Nakamura S, Hiraku Y, Oikawa S, Murata M and Takeuchi K. Circulating microRNAs as novel prognosis biomarkers for head and neck squamous cell carcinoma. Cancer Biol Ther 2015; 16: 1042-1046.
[27] Sun J, Chen Z, Tan X, Zhou F, Tan F, Gao Y, Sun N, Xu X, Shao K and He J. MicroRNA-99a/100 promotes apoptosis by targeting mTOR in human esophageal squamous cell carcinoma. Med Oncol 2013; 30: 411.
[28] Zhou H, Tang K, Xiao H, Zeng J, Guan W, Guo X, Xu H and Ye Z. A panel of eight-miRNA signature as a potential biomarker for predicting survival in bladder cancer. J Exp Clin Cancer Res 2015; 34: 53.
[29] Xu Z, Yu YQ, Ge YZ, Zhu JG, Zhu M, Zhao YC, Xu LW, Yang XB, Geng LG, Dou QL and Jia RP. MicroRNA expression profiles in muscle-invasive bladder cancer: identification of a four-microRNA signature associated with patient survival. Tumour Biol 2015; 36: 8159-8166.
[30] Li D, Liu X, Lin L, Hou J, Li N, Wang C, Wang P, Zhang Q, Zhang P, Zhou W, Wang Z, Ding G, Zhuang SM, Zheng L, Tao W and Cao X. MicroRNA-99a inhibits hepatocellular carcinoma growth and correlates with prognosis of patients with hepatocellular carcinoma. J Biol Chem 2011; 286: 36677-36685.
[31] Petroulakis E, Mamane Y, Le Bacquer O, Shahbazian D and Sonenberg N. mTOR signaling: implications for cancer and anticancer therapy. Br J Cancer 2007; 96 Suppl: R11-15.
[32] Yen YC, Shiah SG, Chu HC, Hsu YM, Hsiao JR, Chang JY, Hung WC, Liao CT, Cheng AJ, Lu YC and Chen YW. Reciprocal regulation of microRNA-99a and insulin-like growth factor I receptor signaling in oral squamous cell carcinoma cells. Mol Cancer 2014; 13: 6.
[33] Oneyama C, Ikeda J, Okuzaki D, Suzuki K, Kanou T, Shintani Y, Morii E, Okumura M, Aozasa K and Okada M. MicroRNA-mediated downregulation of mTOR/FGFR3 controls tumor growth induced by Src-related oncogenic pathways. Oncogene 2011; 30: 3489-3501.
[34] Sun D, Lee YS, Malhotra A, Kim HK, Matecic M, Evans C, Jensen RV, Moskaluk CA and Dutta A. miR-99 family of MicroRNAs suppresses the expression of prostate-specific antigen and prostate cancer cell proliferation. Cancer Res 2011; 71: 1313-1324.
[35] Raman K and Kuberan B. Chemical Tumor Biology of Heparan Sulfate Proteoglycans. Curr Chem Biol 2010; 4: 20-31.
[36] Naiki T, Saijou E, Miyaoka Y, Sekine K and Miyajima A. TRB2, a mouse Tribbles ortholog, suppresses adipocyte differentiation by inhibiting AKT and C/EBP beta. J Biol Chem 2007; 282: 24075-24082.
[37] Xin JX, Yue Z, Zhang S, Jiang ZH, Wang PY, Li YJ, Pang M and Xie SY. miR-99 inhibits cervical carcinoma cell proliferation by targeting TRIB2. Oncol Lett 2013; 6: 1025-1030.
[38] Myatt SS and Lam EW. The emerging roles of forkhead box (Fox) proteins in cancer. Nat Rev Cancer 2007; 7: 847-859.
[39] Cho EC, Kuo ML, Liu X, Yang L, Hsieh YC, Wang J, Cheng Y and Yen Y. Tumor suppressor FOXO3 regulates ribonucleotide reductase subunit RRM2B and impacts on survival of cancer patients. Oncotarget 2014; 5: 4834-4844.