

The impact of miR-9 in osteosarcoma

A study based on meta-analysis, TCGA data, and bioinformatics analysis

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Abstract

The function of miR-9 in osteosarcoma is not well-investigated and controversial. Therefore, we conducted meta-analysis to explore the role of miR-9 in osteosarcoma, and collected relevant TCGA data to further testify the result. In addition, bioinformatics analysis was conducted to investigate the mechanism and related pathways of miR-9-3p in osteosarcoma.

Literature search was operated on databases up to February 19, 2020, including PubMed, Web of Science, Science Direct, Cochrane Central Register of Controlled Trials, and Wiley Online Library, China National Knowledge Infrastructure, China Biology Medicine disc, Chongqing VIP, and Wan Fang Data. The relation of miR-9 expression with survival outcome was estimated by hazard ratio (HRs) and 95% CIs. Meta-analysis was conducted on the Stata 12.0 (Stata Corporation, TX). To further assess the function of miR-9 in osteosarcoma, relevant data from the TCGA database was collected. Three databases, miRDB, miRPathDB 2.0, and Targetscan 7.2, were used for prediction of target genes. Genes present in these 3 databases were considered as predicted target genes of miR-9-3p. Venny 2.1 were used for intersection analysis. Subsequently, GO, KEGG, and PPI network analysis were conducted based on the overlapping target genes of miR-9-3p to explore the possible molecular mechanism in osteosarcoma.

Meta-analysis shown that overexpression of miR-9 was associated with worse overall survival (OS) (HR = 4.180, 95% CI: 2.880–6.066, $P < .001$, $I^2 = 23.5\%$). Based on TCGA data, osteosarcoma patients with overexpression of miR-9-3p (HR = 1.603, 95% CI: 1.028–2.499, $P = .037$) and miR-9-5p (HR = 1.698, 95% CI: 1.133–2.545, $P = .01$) also suffered poor OS. In bioinformatics analysis, 2 significant and important pathways were enriched: Wnt signaling pathway from gene ontology analysis (gene ontology:0016055, P -adjust = .008); hippo signaling pathway from Kyoto Encyclopedia of Genes and Genomes analysis (P -adjust = .007). Moreover, network analysis relevant protein-protein interaction was visualized, revealing 117 nodes and 161 edges.

High miR-9 expression was associated with poor prognosis. Based on bioinformatics analysis, this study enhanced the understanding of the mechanism and related pathways of miR-9 in osteosarcoma.

Abbreviations: GO = gene ontology, HR = hazard ratio, KEGG = Kyoto Encyclopedia of Genes and Genomes, MF = molecular function, miRNA = microRNA, OR = odds ratio, OS = overall survival, PPI = protein-protein interaction, TCGA = The Cancer Genome Atlas.

Keywords: microRNA-9, osteosarcoma, meta-analysis, the cancer genome atlas, bioinformatics

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1. Introduction

Osteosarcoma is the most frequent type of primary malignant bone tumor among young population, which characterized by the deposition of malignant osteoid because of the malignant proliferation of primitive mesenchymal stem cells.^[1,2] The incidence of osteosarcoma is 3.4 cases per million people per year in the world.^[3] Nowadays, the most effective treatment for osteosarcoma is the combination of surgical resection and chemotherapy,^[4,5] which increased the 5-year survival rate to 80%.^[6] Although the overall survival of osteosarcoma patients has improved, cases with metastatic or recurrent disease have a poor prognosis.^[7] Hence, it is necessary to explore the causative factors to improve the therapeutic strategies, which improve the outcome of patients with osteosarcoma.

MicroRNA (miRNA) is a kind of noncoding RNA with a length of about 22 nucleotides.^[8,9] Some publications showed that miRNA can regulate transcription and translation.^[10,11] Furthermore, miRNA also plays a vital role in cell proliferation, differentiation, and apoptosis.^[12,13] Several miRNAs have been proved that associated with poor prognosis in osteosarcoma patients.^[14–16] Currently, studies found that miRNA-9 (miR-9) is downregulated in gastric cancer,^[17] breast cancer,^[18] and renal cell carcinoma^[19] because of promoter methylation; however, miR-9 has been found to be upregulated in colorectal cancer^[20] and gliomas.^[21] Until now, the function of miR-9 in osteosarcoma is not well-investigated, and it is unclear the detail mechanism of miR-9 expression contributes to osteosarcoma pathogenesis.

To explore the expression and potential biological processes of miR-9 in osteosarcoma, we conducted meta-analysis to explore the role of miR-9 in osteosarcoma, and collected relevant TCGA data to further testify the result. In addition, bioinformatics analyses including Gene ontology (GO), Kyoto Encyclopedia of Genes and Genomes (KEGG) and the protein-protein interaction (PPI) network analysis were conducted based on the overlapping target genes of miR-9-3p to explore the possible molecular mechanism in osteosarcoma.

2. Materials and methods

2.1. Meta-analysis

This was conducted by reviewing previous papers, thereby ethical approval is not required. Literature search was operated on databases up to February 19, 2020, including PubMed, Web of Science, Science Direct, Cochrane Central Register of Controlled Trials, and Wiley Online Library, China National Knowledge Infrastructure, China Biology Medicine disc, Chongqing VIP, and Wan Fang Data. The keywords were as follows: (osteosarcoma OR osteogenic sarcoma OR osteogenic tumor) and (miR-9 or miRNA-9 or microRNA-9 or miR9 or miRNA9 or microRNA9 or “miR 9” or “miRNA 9” or “microRNA 9” or miR-9 or miRNA-9 or microRNA-9 or miR-9 or miRNA-9 or microRNA-9).

The included criteria were:

- (1) the relation of miR-9 expression and survival outcome or clinicopathological parameters was evaluated;
- (2) hazard ratio (HR) or sufficient row data was available to calculate HR;

The excluded criteria were:

- (1) review, experimental studies, conference abstracts, expert opinion, or case report;

- (2) miR-9 was combined with other factors to calculate a HR. The following data was extracted: first author, publish year, country, tumor type, sample size, sample source, test method, follow-up time, survival index, statistical method, clinicopathological parameters, and HR and its 95% CIs. Newcastle-Ottawa quality assessment scale was used to assess the quality of included studies. The relation of miR-9 expression with survival outcome was estimated by HRs and 95% CIs. HR less than 1 with 95% CI not overlapping 1 indicates a better prognosis for the miR-9 high expression group. We used the methods described by Parmar et al and the software Engauge Digitizer Version4.1 (<http://digitizer.sourceforge.net/>) when the Kaplan–Meier survival curve was the only available data.^[22] When Kaplan–Meier survival, multivariate and/or univariate analyses were all available, the multivariate HRs and 95% CIs were combined for better revealing the effect of multiple factors on the survival response, follow by univariate analysis, and Kaplan–Meier survival curve. Considering many sources of heterogeneity among the studies and consequently among their individual HR, we calculated the overall HR with a random-effect model.^[23] The odds ratio (OR) with 95% CI were used to assess clinicopathological parameters and heterogeneity was tested by Q-statistic and I²-statistic, I² > 50% was considered to be significant heterogeneity and the random-effects model was used, otherwise the fixed-effects model. Meta-analysis was conducted on the Stata 12.0 (Stata Corporation, TX).

2.2. TCGA data collection

To further assess the function of miR-9 in osteosarcoma, relevant data from the TCGA database (<https://cancergenome.nih.gov/>) was collected. Overall survival (OS), disease-free survival were all analyzed by univariate Cox regression, and the relation between miR-9 and the clinicopathological parameters was assessed by the Independent-sample *t* test. The statistical analysis was conducted on SPSS statistical software package, version 21.0 (IBM Corporation, Armonk, NY), and *P* < .05 indicates significant.

2.3. Bioinformatic analysis

Three databases, miRDB,^[24] miRPathDB 2.0,^[25] and TargetsScan 7.2,^[26] were used for prediction of target genes. Genes present in these 3 databases were considered as predicted target genes of miR-9-3p. Online tool, Venny 2.1 (<https://bioinfogp.cnb.csic.es/tools/venny/>), were used for intersection analysis. Subsequently, GO, KEGG, and PPI network analysis were conducted based on the overlapping target genes of miR-9-3p to explore the possible molecular mechanism in osteosarcoma. GO can describe the functions of gene products from all organisms.^[27] GO annotation includes 3 categories: biological process, cellular component and molecular function. KEGG is a comprehensive knowledge base for both functional interpretation and practical application of genomic information.^[28] In the present study, GO and KEGG analyses were conducted by the clusterProfiler package of R software.^[29] *P* value < .05 and *Q* values < 0.05 were set as the cutoff criterion.^[30,31] Additionally, the PPI network was analyzed by the search tool for the retrieval of interacting genes database (<https://string-db.org/>), and target genes with target score ≥ 80 were used for PPI analysis. The cut-off criteria were a combined score of > 0.9 for a PPI network. Disconnected nodes

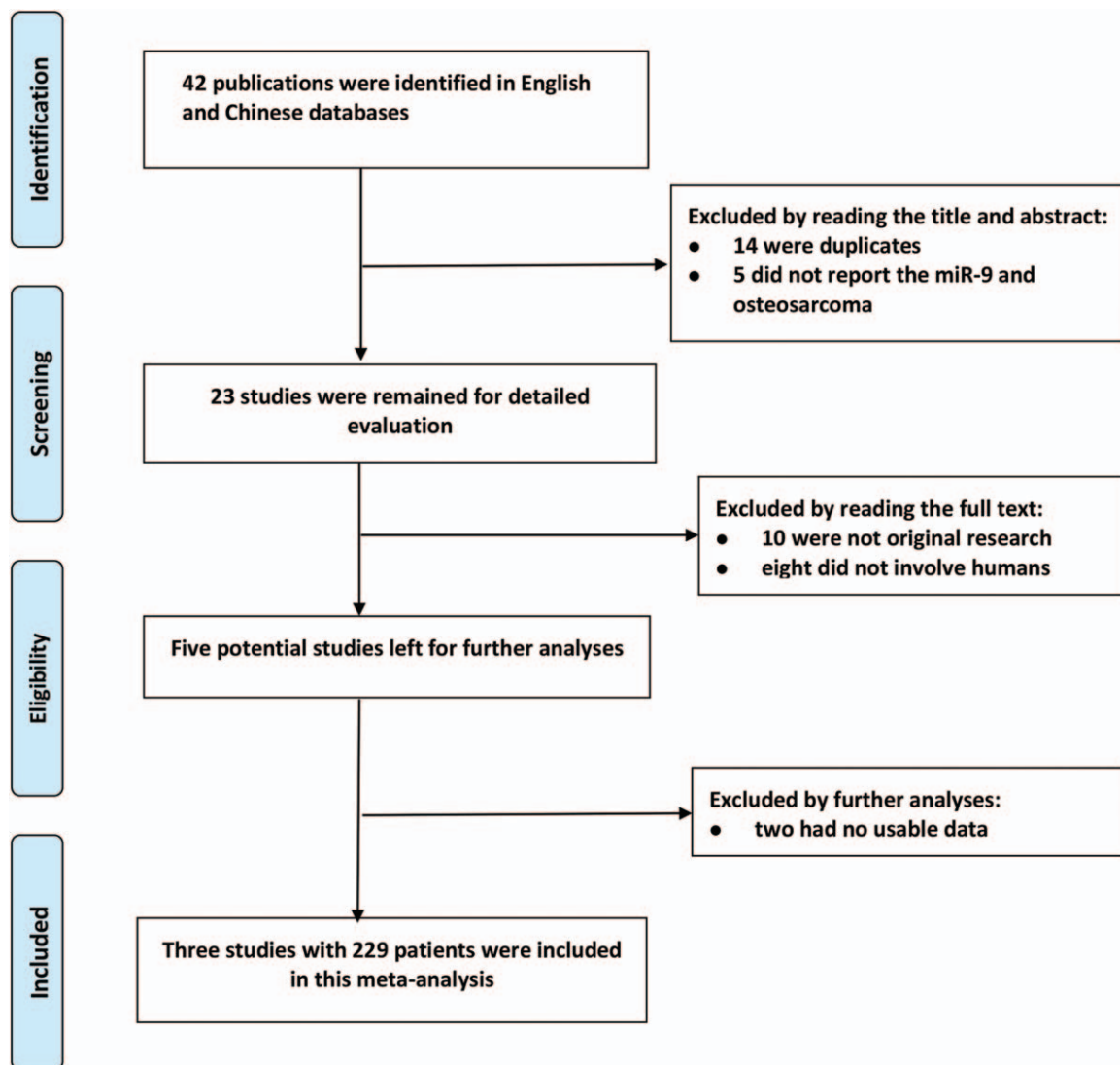


Figure 1. The detailed process of literature research.

were hidden in the network. Line thickness indicates the strength of data support. Furthermore, we used the plug-in of molecular complex detection (MCODE) app in Cytoscape 3.7.0 software to extract hub genes from the PPI network.^[32] The advanced options set as degree cutoff=2, K-Core=2, and Node Score Cutoff=0.2.

3. Results

3.1. Meta-Analysis

The detailed process of literature research was shown in Figure 1. Three studies published from 2014 to 2015 with 229 patients were included in the meta-analysis.^[33–35] Based on the expression level of miR-9, 2 studies evaluated overall-survival (OS)^[34,35] and the rest 1 only evaluated clinicopathological parameters.^[33] The characteristics and quality score of the included studies were shown in Table 1. Meta-analysis shown that high expression of miR-9 was associated with worse OS for osteosarcoma patients, the combined HR was 4.180 (95% CI:

2.880–6.066, $P < .001$, $I^2 = 23.5\%$) (Fig. 2A). However, there was no statistical significance between the expression of miR-9 and clinicopathological parameters, including gender (OR = 1.729, 95% CI: 0.811–3.683, $P = .156$, $I^2 = 0\%$) (Fig. 2B) and stage (OR = 3.629, 95% CI: 0.318–41.364, $P = .299$, $I^2 = 83.3\%$) (Fig. 2C).

3.2. Analyses on TCGA data

215 and 260 osteosarcoma patients with miR-9-3p and miR-9-5p expression levels were downloaded from TCGA database. Univariate Cox regression shown that overexpression of miR-9-3p (HR = 1.603, 95% CI: 1.028–2.499, $P = .037$) (Fig. 3A) and miR-9-5p (HR = 1.698, 95% CI: 1.133–2.545, $P = 0.01$) (Fig. 3B) were all linked with better OS but not with disease-free survival (HR = 0.837, 95% CI: 0.470–1.493, $P = .548$ for miR-9-3p; HR = 1.090, 95% CI: 0.668–1.778, $P = .732$ for miR-9-5p). Interesting, female patients tended to have higher miR-9-3p ($P = .024$) and miR-9-5p ($P = .006$) expressions (Table 2).

Table 1
The characteristics and quality score of included studies.

First author	Yr	Country	Sample source	Test method	Case	Follow-up (mo)	Survival	Statistic method	HR	LL	UL	Outcome	Gender		Stage		NOS				
													High expression	Low expression	High expression	Low expression					
Fei D	2014	China	Serum	qRT-PCR	118	48	OS	Survival curve	3.18	1.35	4.42	Worse	/	/	/	/	/	/	7		
Xu S	2014	China	Tissue	qRT-PCR	79	60	OS	Multivariate analysis	4.77	2.86	5.91	Worse	25	15	19	20	31	9	9	30	6
You JN	2015	China	Tissue	qRT-PCR	32	/	/	/	/	/	/	/	8	6	8	10	3	11	4	14	5

* = outcome was for patient with high miR-9 expression, / = not available, HR = hazard ratio, LL = lower limit, NOS = the scores of Newcastle-Ottawa quality assessment scale, OS = overall survival, UL = upper limit.

3.3. Bioinformatic analysis

In total, 991 genes were predicted from miRDB, 6649 genes were predicted from miRPathDB, 4306 genes were predicted from Targetscan, and finally 909 overlapping target genes were obtained. In bioinformatics analysis based on the overlapping target genes, 2 significant and important pathways were enriched: Wnt signaling pathway from GO analysis (GO:0016055, *P*-adjust = .008); hippo signaling pathway from KEGG analysis (*P*-adjust = .007). Meanwhile, based on adjusted *P* value and genes count, gland development (GO:0048732), forebrain development (GO:0030900), and positive regulation of cell adhesion (GO:0045785) were the top 3 pathways in the biological process. With respect to cellular component, the top 3 terms gathered by

these target genes were neuronal cell body (GO:0043025), synaptic membrane (GO:0097060), and cell to cell junction (GO:0005911). These target genes were also significantly clustered in terms of molecular function, such as proximal promoter sequence to specific DNA binding (GO:0000987), RNA polymerase II proximal promoter sequence to specific DNA binding (GO:0000978), and transcription coregulator activity (GO:0003712) (Figs. 4 and 5A, and Supplementary Table 1, <http://links.lww.com/MD/E748>). With regard to KEGG pathway analysis, the 9 significant signaling pathways for the target genes of miR-9-3p were hepatocellular carcinoma, hippo signaling pathway, longevity regulating pathway, tight junction, chronic myeloid leukemia, signaling pathways regulating

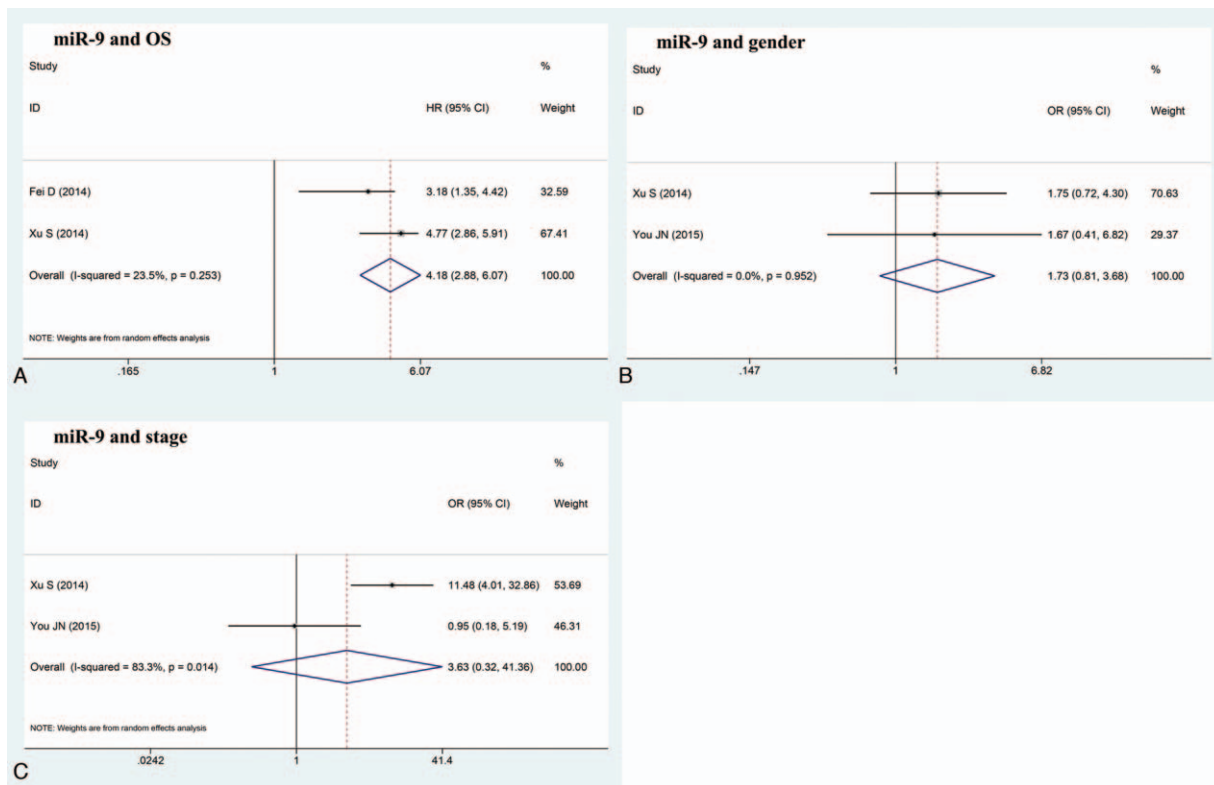


Figure 2. The forest plots for the relation between miR-9 and (A) OS (HR=4.180, 95% CI: 2.880-6.066, *P* < .001, *I*² = 23.5%), (B) gender (OR = 1.729, 95% CI: 0.811-3.683, *P* = .156, *I*² = 0%) and (C) stage (OR = 3.629, 95% CI: 0.318-41.364, *P* = .299, *I*² = 83.3%).

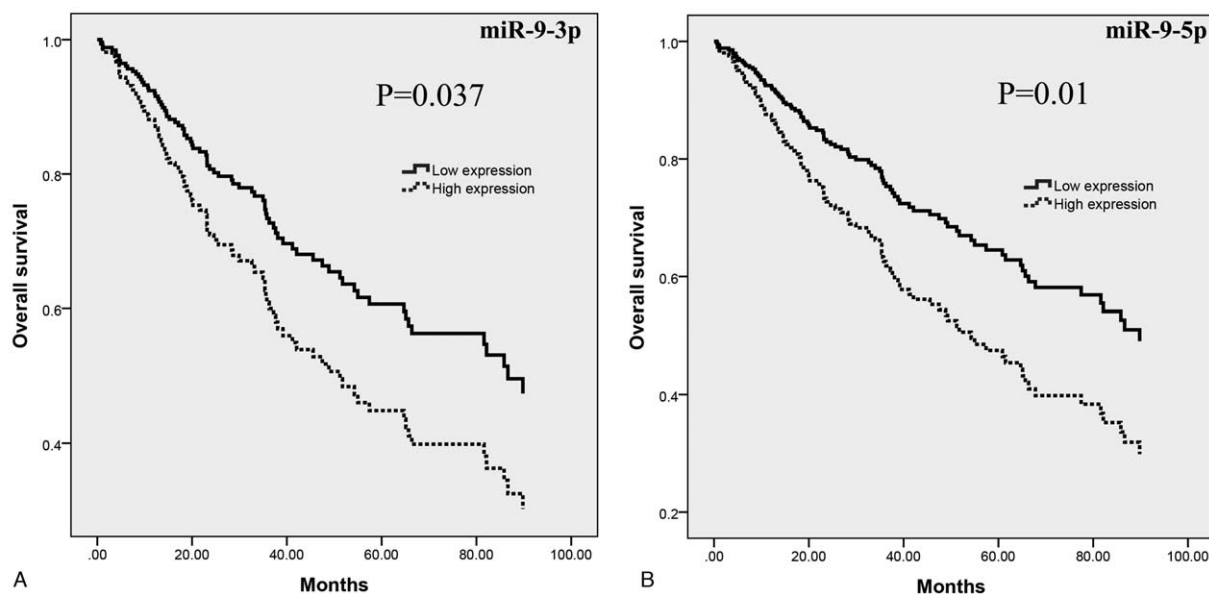


Figure 3. (A) Overall survival analysis of miR-9-3p ($P=.037$), and (B) miR-9-5p ($P=.01$) in osteosarcoma.

pluripotency of stem cells, foxO signaling pathway, melanoma, and non-small cell lung cancer (all P and Q values $< .05$, Fig. 5B, Supplementary Table 2, <http://links.lww.com/MD/E749>). Furthermore, molecular pathways and processes were calculated on 348 genes to generate a PPI network (Fig. 6). Relevant PPI were visualized, revealing 117 nodes and 161 edges.

4. Discussion

In the meta-analysis, we confirmed that patients with high miR-9 expression had poor OS, and based on TCGA data, we also found that miR-9-3p and miR-9-5p were associated with worse OS. Among included studies, Fei et al reported that serum miR-9

levels of osteosarcoma patients were significantly increased, compared to the healthy controls, and upregulation of miR-9 was associated with worse OS, advanced stage, larger tumor size and positive metastasis status.^[35] The results of Xu et al study indicated that high miR-9 expression was strongly correlated with aggressive progression of osteosarcoma and unfavorable OS.^[34] Although meta-analysis showed that miR-9 was not associated with osteosarcoma stage, it is worth mentioning that the heterogeneity between 2 included studies upped to 83.3%. The study of Xu et al with 79 patients indicated high expression of miR-9 was linked with advanced osteosarcoma stage ($P=.009$),^[34] but You et al reported that miR-9 expression was not associated with stage based on only 32 patients ($P=.7$).^[33] Therefore, more patients are needed to verify the

Table 2

The relation of miR-9 expression and clinicopathological parameters of osteosarcoma (TCGA data).

Clinicopathological parameters	Case	miR-9-3p			P	Case	miR-9-5p		
		Expression		P			Expression		P
		Mean	SD				Mean	SD	
Age									
<60 yr	99	1.76	1.48	.189	117	9.35	2.49	.623	
≥60 yr	116	1.50	1.37		143	9.20	2.36		
Gender									
Female	120	1.81	1.48	.024	141	9.6439	2.44147	.006	
Male	95	1.38	1.31		119	8.8256	2.31751		
Recurrence									
Yes	23	1.85	1.53	.394	29	9.3341	2.33542	.905	
No	125	1.55	1.37		144	9.2771	2.31436		
Metastasis									
Yes	49	1.63	1.29	.958	59	9.18	2.40	.539	
No	101	1.64	1.46		118	9.41	2.29		
Tumor depth									
<5 cm	62	1.58	1.44	.976	79	9.21	2.35	.841	
≥5 cm	89	1.57	1.43		106	9.14	2.54		

SD=standard deviation.

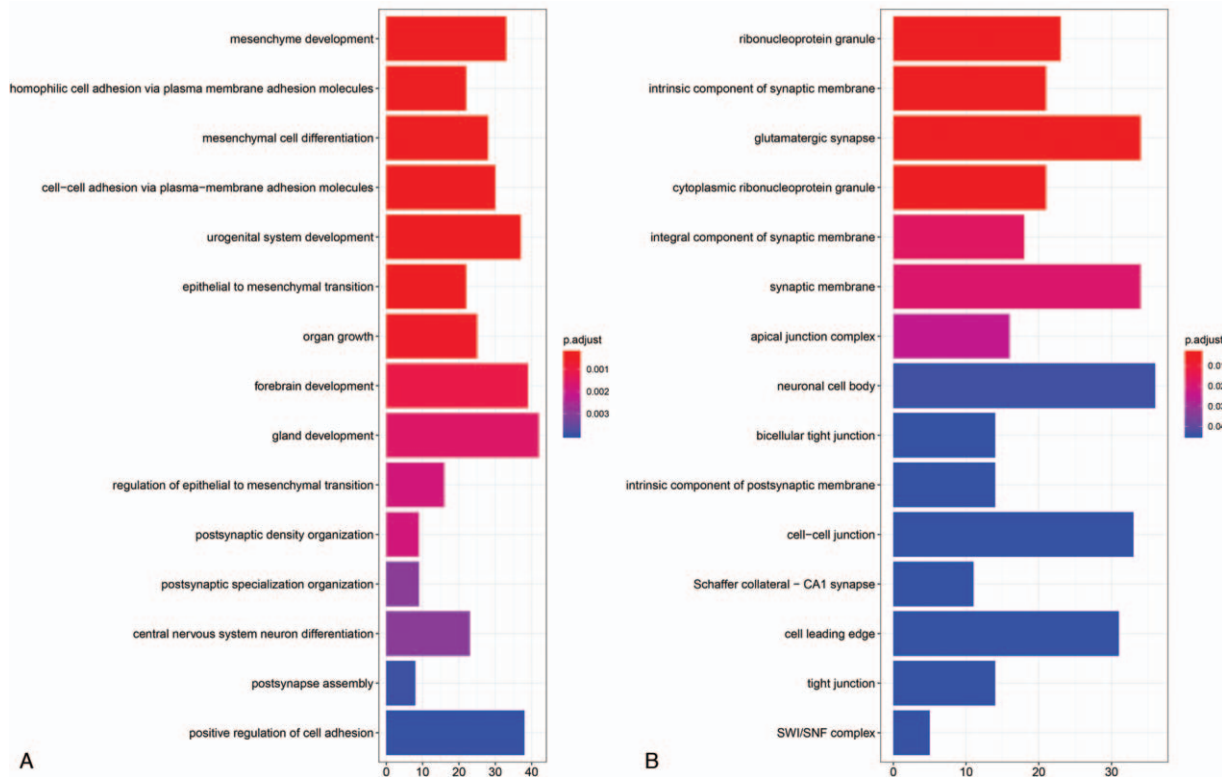


Figure 4. Bar plots of gene ontology (GO) analysis. (A)GO_biological process, (B) GO_cellular component.

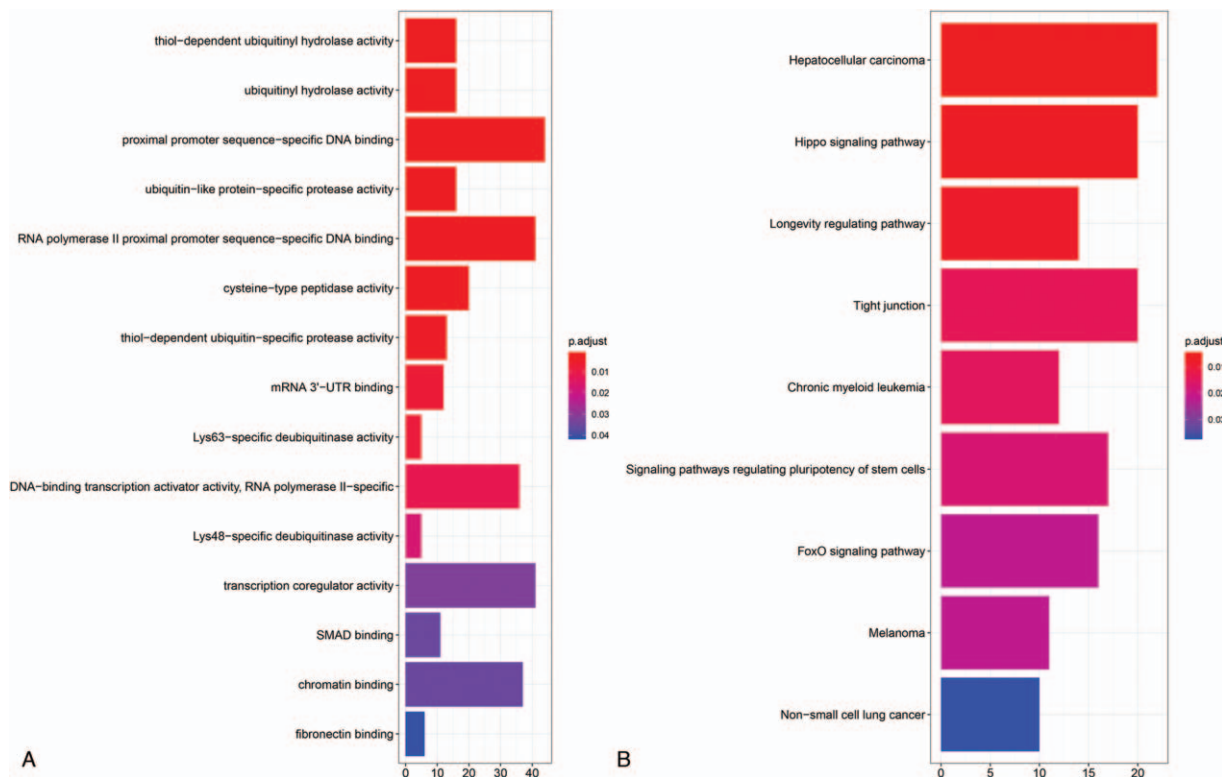


Figure 5. Bar plots of gene ontology and Kyoto Encyclopedia of Genes and Genome analysis. (A) gene ontology_molecular function, (B) Kyoto Encyclopedia of Genes and Genome.

cancer, miR-9 up-regulation is involved metastasis by promoting cell motility,^[20] and was identified upregulated in gliomas.^[21] In osteosarcoma, inhibition of miR-9 decreases the proliferation of tumor cells by targeting p16.^[39,40] In addition, miR-9 was upregulated in spontaneous canine osteosarcoma and promoted the invasion and migration of osteosarcoma cells.^[41] Meanwhile, miR-9 was an oncogene and promoted proliferation of osteosarcoma through targeting Grap2 and cyclin D interacting protein.^[42] Upregulated miR-9 expression was also related with increased cell proliferation, migration, invasion, and decreased apoptotic ability.^[43]

In bioinformatics analysis, 2 significant and important pathways were enriched: Wnt signaling pathway from GO analysis and hippo signaling pathway from KEGG. Many studies found that Wnt signaling pathway plays an important role in the development of osteosarcoma. Du et al.^[44] found that inhibition of miRNA-184 may reduce tumor volume of osteosarcoma by regulating the Wnt/ β -catenin signaling pathway. Zhang et al.^[45] suggested that miR-107 inhibit development of osteosarcoma via the Wnt signaling pathway, and the critical role of miR-214 in human osteosarcoma also through involving the Wnt signaling pathway.^[46] Hence, it was proposed that miR-9-3p may possess a vital effect on osteosarcoma by Wnt signaling pathway. KEGG analysis of the target genes of the miR-9-3p showed hippo signaling pathway is a significant pathway, which associated with worse prognostic outcomes in various cancers. Furthermore, many studies have demonstrated that hippo signaling pathway is an important pathway in osteosarcoma.^[47–49] Previous study found that defects in the hippo signaling pathway induce the hyperactivation of its downstream effector like transcriptional co-activator with PDZ-binding motif (TAZ).^[50] Higashi et al verified that miR-9-3p targeting TAZ expression in hepatocellular carcinoma.^[51] Taken together, above results suggested that miR-9-3p might act as an efficient regulator for osteosarcoma development.

Although our study confirmed that osteosarcoma patients with increased miR-9 suffered poor prognosis, several limitations should be mentioned in our study. Firstly, the heterogeneity in meta-analysis of stage was significant, which might generate from the different characteristics, treatments, and cut-off values of patients. Secondly, results of clinicopathological parameter analysis were different in meta-analysis and TCGA data analysis. That may be caused by small sample size and included studies in meta-analysis. The number of patients was small and included studies were limited to China in the meta-analysis. However, a larger number of patients were from USA in the TCGA data analysis. Thus, our results need to be confirmed by more patients from different countries. Thirdly, only survival curve was available in the study of Fei et al,^[35] this might bring bias when we obtained HR data. Finally, all included studies were retrospective studies, which might more inclined to be published with positive result rather than negative one. Thus, the relation between overexpression of miR-9 and poor prognosis in osteosarcoma might be overestimated.

5. Conclusion

Our study revealed that high miR-9 expression was associated with poor prognosis, compared with low expression of miR-9. Based on bioinformatics analysis, this study enhanced the understanding of the mechanism and related pathways of miR-9 in osteosarcoma.

Author contributions

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References

- [1] Biermann JS, Adkins DR, Agulnik M, et al. Bone cancer. *J Natl Compr Canc Netw* 2013;11:688–723.
- [2] Zhang Y, Yang J, Zhao N, et al. Progress in the chemotherapeutic treatment of osteosarcoma. *Oncol Lett* 2018;16:6228–37.
- [3] Misaghi A, Goldin A, Awad M, et al. Osteosarcoma: a comprehensive review. *SICOT J* 2018;4:12.
- [4] Zhou Y, Lu Q, Xu J, et al. The effect of pathological fractures on the prognosis of patients with osteosarcoma: a meta-analysis of 14 studies. *Oncotarget* 2017;8:73037–49.
- [5] Wong KC, Lee V, Shing MM, et al. Surgical resection of relapse may improve postrelapse survival of patients with localized osteosarcoma. *Clin Orthop Relat Res* 2013;471:814–9.
- [6] Bacci G, Ruggieri P, Bertoni F, et al. Local and systemic control for osteosarcoma of the extremity treated with neoadjuvant chemotherapy and limb salvage surgery: the Rizzoli experience. *Oncol Rep* 2000;7:1129–33.
- [7] Meazza C, Scanagatta P. Metastatic osteosarcoma: a challenging multidisciplinary treatment. *Expert Rev Anticancer Ther* 2016;16:543–56.
- [8] Llave C, Xie Z, Kasschau KD, et al. Cleavage of Scarecrow-like mRNA targets directed by a class of *Arabidopsis* miRNA. *Science (New York, NY)* 2002;297:2053–6.
- [9] Fire A, Xu S, Montgomery MK, et al. Potent and specific genetic interference by double-stranded RNA in *Caenorhabditis elegans*. *Nature* 1998;391:806–11.
- [10] Filipowicz W, Bhattacharyya SN, Sonenberg N. Mechanisms of post-transcriptional regulation by microRNAs: are the answers in sight? *Nat Rev Genet* 2008;9:102–14.
- [11] Valencia-Sanchez MA, Liu J, Hannon GJ, et al. Control of translation and mRNA degradation by miRNAs and siRNAs. *Genes Dev* 2006;20:515–24.
- [12] Hwang HW, Mendell JT. MicroRNAs in cell proliferation, cell death, and tumorigenesis. *Br J Cancer* 2006;94:776–80.
- [13] Jovanovic M, Hengartner MO. miRNAs and apoptosis: RNAs to die for. *Oncogene* 2006;25:6176–87.
- [14] Sarver AL, Thayanithy V, Scott MC, et al. MicroRNAs at the human 14q32 locus have prognostic significance in osteosarcoma. *Orphanet J Rare Dis* 2013;8:7.
- [15] Arabi L, Gsponer JR, Smida J, et al. Upregulation of the miR-17-92 cluster and its two paralogs in osteosarcoma - reasons and consequences. *Genes Cancer* 2014;5:56–63.
- [16] Wang W, Zhou X, Wei M. MicroRNA-144 suppresses osteosarcoma growth and metastasis by targeting ROCK1 and ROCK2. *Oncotarget* 2015;6:10297–308.
- [17] Tsai KW, Liao YL, Wu CW, et al. Aberrant hypermethylation of miR-9 genes in gastric cancer. *Epigenetics* 2011;6:1189–97.
- [18] Lehmann U, Hasemeier B, Christgen M, et al. Epigenetic inactivation of microRNA gene hsa-mir-9-1 in human breast cancer. *J Pathol* 2008;214:17–24.

- [19] Hildebrandt MA, Gu J, Lin J, et al. Hsa-miR-9 methylation status is associated with cancer development and metastatic recurrence in patients with clear cell renal cell carcinoma. *Oncogene* 2010;29:5724–8.
- [20] Zhu L, Chen H, Zhou D, et al. MicroRNA-9 up-regulation is involved in colorectal cancer metastasis via promoting cell motility. *Med Oncol* (Northwood, London, England) 2012;29:1037–43.
- [21] Malzkorn B, Wolter M, Liesenberg F, et al. Identification and functional characterization of microRNAs involved in the malignant progression of gliomas. *Brain Pathol* (Zurich, Switzerland) 2010;20:539–50.
- [22] Parmar MK, Torri V, Stewart L. Extracting summary statistics to perform meta-analyses of the published literature for survival endpoints. *Stat Med* 1998;17:2815–34.
- [23] Riley RD, Elia EG, Malin G, et al. Multivariate meta-analysis of prognostic factor studies with multiple cut-points and/or methods of measurement. *Stat Med* 2015;34:2481–96.
- [24] Liu W, Wang X. Prediction of functional microRNA targets by integrative modeling of microRNA binding and target expression data. *Genome Biol* 2019;20:18.
- [25] Backes C, Kehl T, Stockel D, et al. miRPathDB: a new dictionary on microRNAs and target pathways. *Nucleic Acids Res* 2017;45:D90–6.
- [26] Agarwal V, Bell GW, Nam JW, et al. Predicting effective microRNA target sites in mammalian mRNAs. *Elife* 2015;4:e05005.
- [27] Thomas PD. The gene ontology and the meaning of biological function. *Methods Mol Biol* (Clifton, NJ) 2017;1446:15–24.
- [28] Kanehisa M, Furumichi M, Tanabe M, et al. KEGG: new perspectives on genomes, pathways, diseases and drugs. *Nucleic Acids Res* 2017;45: D353–61.
- [29] Yu G, Wang LG, Han Y, et al. ClusterProfiler: an R package for comparing biological themes among gene clusters. *OMICS* 2012; 16:284–7.
- [30] Chen X, Cai S, Wang L, et al. Analysis of the function of MAGE-A in esophageal carcinoma by bioinformatics. *Medicine* 2019;98:e15774.
- [31] Chumbley JR, Friston KJ. False discovery rate revisited: FDR and topological inference using Gaussian random fields. *Neuroimage* 2009;44:62–70.
- [32] Bader GD, Hogue CW. An automated method for finding molecular complexes in large protein interaction networks. *BMC Bioinformatics* 2003;4:2.
- [33] You JN. Expression of miR-9 in osteosarcoma and its clinical relevance. *Chin J Lab Diagn* 2015;19:27–9.
- [34] Xu SH, Yang YL, Han SM, et al. MicroRNA-9 expression is a prognostic biomarker in patients with osteosarcoma. *World J Surg Oncol* 2014; 12:195.
- [35] Fei D, Li Y, Zhao D, et al. Serum miR-9 as a prognostic biomarker in patients with osteosarcoma. *J Int Med Res* 2014;42:932–7.
- [36] Yang L, Mu Y, Cui H, et al. MiR-9-3p augments apoptosis induced by H₂O₂ through down regulation of herpud1 in glioma. *PLoS One* 2017;12:e0174839.
- [37] Li DY, Chen WJ, Shang J, et al. Regulatory interactions between long noncoding RNA LINC00968 and miR-9-3p in non-small cell lung cancer: a bioinformatic analysis based on miRNA microarray. *GEO and TCGA Oncol Lett* 2018;15:9487–97.
- [38] Li DY, Chen WJ, Luo L, et al. Prospective lncRNA-miRNA-mRNA regulatory network of long non-coding RNA LINC00968 in non-small cell lung cancer A549 cells: a miRNA microarray and bioinformatics investigation. *Int J Mol Med* 2017;40:1895–906.
- [39] Gao S, Wang J, Tian S, et al. miR9 depletion suppresses the proliferation of osteosarcoma cells by targeting p16. *Int J Oncol* 2019;54:1921–32.
- [40] Gang W, Tanjun W, Yong H, et al. Inhibition of miR-9 decreases osteosarcoma cell proliferation. *Bosn J Basic Med Sci* 2020;20: 218–25.
- [41] Fenger JM, Roberts RD, Iwenofu OH, et al. MiR-9 is overexpressed in spontaneous canine osteosarcoma and promotes a metastatic phenotype including invasion and migration in osteoblasts and osteosarcoma cell lines. *BMC Cancer* 2016;16:784.
- [42] Zhu SW, Li JP, Ma XL, et al. miR-9 modulates osteosarcoma cell growth by targeting the gcip tumor suppressor. *Asian Pac J Cancer Prev* 2015;16:4509–13.
- [43] Qi XJ, Wang JF, Wang GD, et al. Pivotal role of microRNA-9 in osteosarcoma tumorigenesis and tumor progression. *Genet Mol Res* 2016;15:
- [44] Du Z, Li F, Wang L, et al. Regulatory effects of microRNA184 on osteosarcoma via the Wnt/betacatenin signaling pathway. *Mol Med Rep* 2018;18:1917–24.
- [45] Zhang ZC, Liu JX, Shao ZW, et al. In vitro effect of microRNA-107 targeting Dkk-1 by regulation of Wnt/beta-catenin signaling pathway in osteosarcoma. *Medicine* 2017;96:e7245.
- [46] Zhu XB, Zhang ZC, Han GS, et al. Overexpression of miR214 promotes the progression of human osteosarcoma by regulating the Wnt/betacatenin signaling pathway. *Mol Med Rep* 2017;15:1884–92.
- [47] Fu CY, Chen MC, Tseng YS, et al. Fisetin activates Hippo pathway and JNK/ERK/AP-1 signaling to inhibit proliferation and induce apoptosis of human osteosarcoma cells via ZAK overexpression. *Environ Toxicol* 2019;34:902–11.
- [48] Luu AK, Schott CR, Jones R, et al. An evaluation of TAZ and YAP crosstalk with TGFbeta signalling in canine osteosarcoma suggests involvement of hippo signalling in disease progression. *BMC Vet Res* 2018;14:365.
- [49] Chai J, Xu S, Guo F. TEAD1 mediates the oncogenic activities of hippo-YAP1 signaling in osteosarcoma. *Biochem Biophys Res Commun* 2017;488:297–302.
- [50] Qin F, Tian J, Zhou D, et al. Mst1 and Mst2 kinases: regulations and diseases. *Cell Biosci* 2013;3:31.
- [51] Higashi T, Hayashi H, Ishimoto T, et al. miR-9-3p plays a tumour-suppressor role by targeting TAZ (WWTR1) in hepatocellular carcinoma cells. *Br J Cancer* 2015;113:252–8.