European Review for Medical and Pharmacological Sciences 2016; 20: 1258-1262

MicroRNA-1908 is a biomarker for poor prognosis in human osteosarcoma

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Abstract. – OBJECTIVE: The aim of the present study was to analyze microRNA-1908 expression in human osteosarcoma and to explore the potential of miRNAs as biomarkers for patient outcomes.

PATIENTS AND METHODS: Real-time quantitative polymerase chain reaction assay was performed to evaluate the expression level of microRNA-1908 in 212 patients diagnosed osteosarcoma. The association of miR-1908 expression with clinicopathological factors or the prognosis of glioma patients were also analyzed. Furthermore, the Kaplan-Meier method and Cox's proportional hazards model was used to determine survival rate.

RESULTS: MiR-1908 expression levels in osteosarcoma tissues were significantly higher than those in matched adjacent normal bone tissues ($p < 0.001$). Tumors with high miR-1908 expression had significantly greater extent of recurrence ($p < 0.003$), metastasis ($p = 0.000$), and chemotherapy response ($p < 0.019$) than those with low miR-1908 expression. Kaplan-Meier analysis showed that patients with low level of miR-1908 had favorable trends of survival ($p < 0.001$). The result of the multivariate analysis showed that miR-1908 expression level and metastasis status retained significance as an independent prognostic factor of human osteosarcoma.

CONCLUSIONS: Our study demonstrates that increased microRNA-1908 expression is associated with poor clinical outcome in human osteosarcoma.

Key Words:
Osteosarcoma, MicroRNA-1908, Biomarker.

Introduction

Osteosarcoma, as the eighth leading cancer with an incidence of 4.4 per million, is the leading cause of cancer-related death among children and adolescents[1]. It is an aggressive bone tumor characterized by malignant osteoid production and malignant cells with osteoblastic differentiation[2]. Although the recent advances in tumor diagnosis and treatment including surgery, radiotherapy, and chemotherapy were tremendous[3]. The incidence of osteosarcoma has been reported to be increasing at a rate of 1.4% per year[4]. The prognosis of patients with recurrent or metastatic OS remains poor[5,6]. Therefore, it is urgent to identify reliable biomarkers of Osteosarcoma for its early diagnosis, effective therapy, and prognosis evaluation.

microRNAs (miRNAs) are endogenously expressed small non-coding RNAs that play a critical role in regulating gene expression[7,8]. MiRNAs bind to target mRNAs to inhibit their translation and/or target them for cleavage and degradation. MiRNA-binding sites are generally located at the 3′-untranslated regions (3′-UTRs) of target mRNAs[9,10]. Emerging evidence demonstrated that aberrant miR expression is highly associated with cancer initiation and progression, which may provide a new promising way to deal with cancer[11-13]. Besides, expression levels of certain miRNAs could serve as a prognostic indicator of survival[14,15]. Therefore, it helps to develop new treatment method and to identify novel miRNAs which are associated with progression of cancer.

MicroRNA-1908 is a newly found miRNA, several lines of evidence have shown that miR-1908 are dysregulated in certain types of human cancer[16,17]. However, the role of miR-1908 in osteosarcoma and its association with prognosis have not been reported.

Patients and Methods

Patients and Tissue Samples

This study was approved by the Ethics Committee of Linyi People Hospital. 212 paired tis-

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sue specimens of osteosarcoma and matched normal tissues were obtained from the Department of Orthopedics in Linyi People Hospital. We performed pathologist to confirm that the matched normal tissues have no tumor cells. Normal laryngeal mucosa specimens were retrieved 10 mm outside the negative margin. Samples were placed in RNAlater Tissue Protect Tubes (Qiagen, Hilden, Germany) and stored at –80°C. All patients did not receive any blood transfusion, radiation treatment, or chemotherapy.

miRNA qRT-PCR Assay

According to the manufacturer's instructions, total RNA from tissue samples was extracted by using Trizol reagent (Invitrogen Inc., Waltham, MA, USA). Primers for miR-1908 and the endogenous control U6 snRNA were obtained from Applied Biosystems (Foster City, CA, USA). The Hairpin-it miRNA qPCR Quantitation Kit (GenePharma, Shanghai, China) was used to analyze the expression level of miR-1908. The PCR conditions were 95°C for 5 min, followed by 40 cycles at 95°C for 15 s, 55°C for 30 s and 72°C for 34 s. Real-time PCR was performed on the SYBR Premix Ex Taq™ II kit (TaKaRa, Japan) on 7500 Real-Time PCR systems (Applied Biosystems, Carlsbad, CA, USA). The universal small nuclear RNA U6 (RNU6B) was used as an endogenous control for miRNAs.

Statistical Analysis

All the statistical analyses were performed using SPSS13.0 for Windows (SPSS Inc., Chicago, IL, USA). Two-sample Student's *t*-test. and chi-square tests analysis or Fisher's exact test were used to analyze the association between miR-1908 expression and various clinicopathological characteristics. Survival curves were done with the Kaplan-Meier method and compared by the log-rank test. Cox proportional hazards regression model was used to explore Prognostic factor of each variable. *p*-values < 0.05 were considered statistically significant.

Results

MiR-1908 was Significantly up-Regulated in Osteosarcoma Samples

qRT-PCR was performed to determine the differential expression of miR-1908 in 212 pairs of osteosarcoma tissues and normal tissues. As shown in Figure 1, our results showed that miR-1908 expression was significantly increased in osteosarcoma tissues, compared with normal tissues ($p < 0.01$).

Correlation of high miR-1908 Expression with Clinicopathological Features of Osteosarcoma

For statistical analysis, miR-1908 expression was divided into high and low expression groups. The correlation between miR-1908 expression with clinicopathological characteristics in patients with osteosarcoma was examined. The high expression of miR-1908 in the osteosarcoma tissues was strongly correlated with the metastasis ($p < 0.001$), poorer chemotherapy response ($p = 0.019$), and a higher recurrence rate ($p = 0.003$). No significant difference was observed between miR-1908 expression and other factors (Table I).

miR-1908 is Prognostic and can be Used as a Predictor of Tumor Recurrence in Osteosarcoma

Kaplan-Meier analysis with log-rank test was performed to examine the effect of miR-1908 expression on OS and DFS. The results showed that the patients with higher miR-1908 expression had both poorer OS ($p < 0.001$) and DFS ($p < 0.001$). as shown in Figure 2. Multivariate analysis confirmed that low miR-1908 (OS $p =$

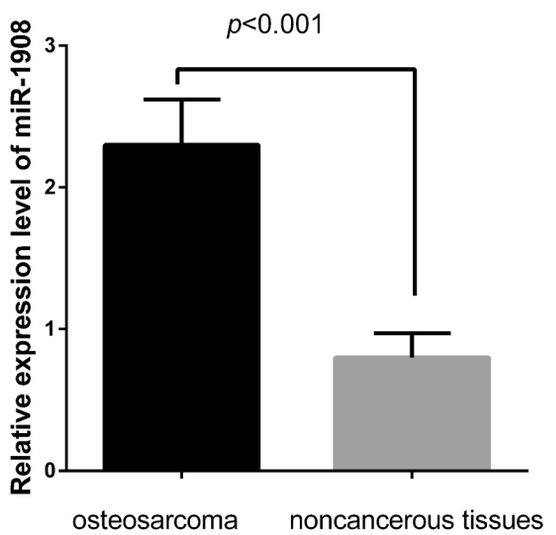

Figure 1. miR-1908 expression was significantly lower in osteosarcoma samples than in the corresponding noncancerous tissues ($p < 0.01$, paired *t*-test).

0.019, DFS $p = 0.008$) expression and metastasis (OS $p = 0.003$, DFS $p = 0.001$) were independent prognostic factors of unfavorable survival in pediatric osteosarcoma (Table II).

Discussion

Although various treatment improvements have been developed for osteosarcoma, including molecular targeted therapy, gene therapy, and immunotherapy. However, the 5-year overall and disease-free survival rates for osteosarcoma patients are around 50-60%[18]. More and more studies have shown that miRNAs may function as a novel group of predictive biomarkers for the management of osteosarcoma. In our study, the prognostic value of miR-1908 in osteosarcoma was explored. To our knowledge, this is the first time to investigate the potential of miRNA-1908 as biomarkers for patient outcomes. Our results showed that miR-1908 was identified as a remarkably increased miRNA in osteosarcoma tissues compared with noncancerous bone tissues ($p < 0.001$) and the high-expression level of miR-1908 was correlation with the metastasis ($p < 0.001$), poorer chemotherapy response ($p = 0.019$), and a higher recurrence rate ($p = 0.003$). Furthermore, Patients with higher miR-1908 expression level had significantly poorer overall survival and disease-free survival, suggesting that miR-1908 expression may function as a potential biomarker for overall survival prediction in patient with osteosarcoma. The results of Cox regression analyses revealed that miR-1908 may be an independent prognostic marker for osteosarcoma patients.

miRNAs are involved in cell differentiation, proliferation, apoptosis, invasion, and distant metastasis[19]. Accumulating studies indicate that aberrant expression of miRNAs is involved in human tumorigenesis and progression[20]. Therefore, exploring the function of individual miRNAs may be help for the treatment of osteosarco-

Table I. Patients and characteristics.

Cases Parameters	miR-1908 expression level High expression (n=212)	Low expression (n=104)	(n=108)	*p*-value
Gender	0.353			
Male	123	57	66	
Female	89	47	42	
Site				0.781
Femur	97	47	52	
Tibia	58	30	28	
Humerus	38	21	17	
Others	17	6	11	
Histologic type				0.512
Osteoblastic	113	52	61	
Chondroblastic	56	29	27	
Fibroblastic	30	15	15	
Telangiectatic	13	8	5	
Tumor grade				0.319
Low	111	45	56	
High	101	59	52	
Resection margin				0.449
Adequate	112	51	61	
Inadequate	100	53	47	
Recurrence				0.003
No	106	41	65	
Yes	106	63	43	
Chemotherapy response			0.019	
Good	105	43	62	
Poor	107	61	46	
Metastasis				0.000
No	107	39	68	
Yes	105	65	40	

Table II. Multivariate survival analysis of overall and disease-free survivals in 212 osteosarcoma patients.

Parameters	Overall survival		Disease-free survival	
	RR (95%CI)	*p*-value	RR (95%CI)	*p*-value
Metastasis	5.21 (1.72-12.59)	0.003	4.22 (1.98-14.19)	0.001
miR-1908 expression	4.41 (1.89-9.66)	0.019	5.16 (1.82-11.92)	0.008

ma. So far, only a few miRNA was identified for osteosarcoma[21]; moreover, sensitivities of those miRNAs are relatively dissatisfactory[22]. Therefore, it is urgent to find out a novel marker of osteosarcoma.

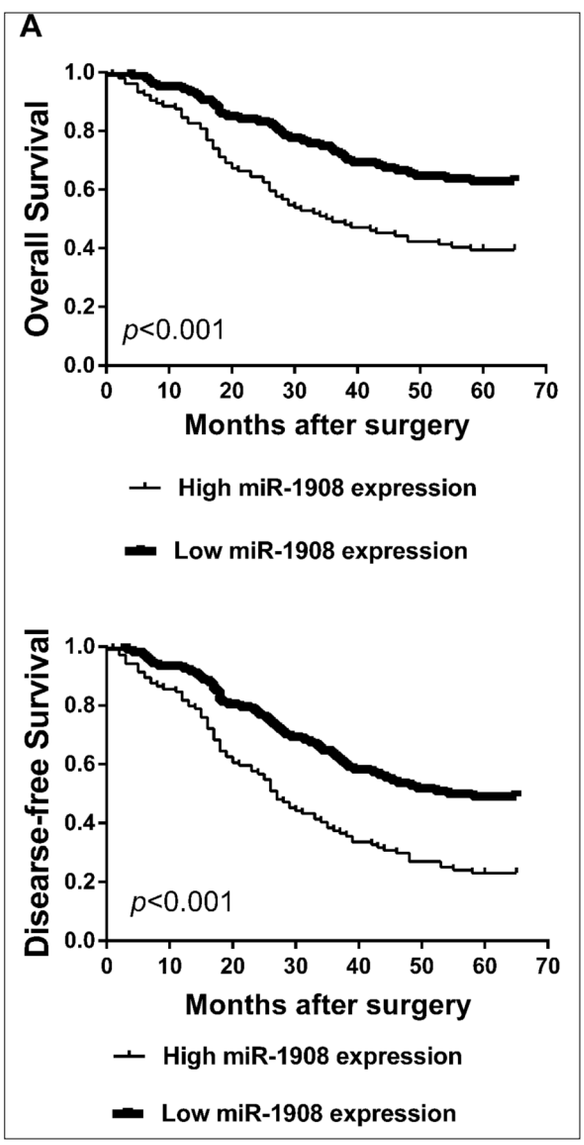

Figure 2. Kaplan-Meier survival curves for osteosarcoma patients according to miR-1908 expression. ***A,*** The patients with higher expression level of miR-1908 had a poorer overall survival rate ($p < 0.001$). ***B,*** The patients with higher expression level of miR-1908 had a poorer disease-free survival rate ($p < 0.001$).

miR-1908 is a new member of the microRNA family. there is little reports about the effect of miRNA-1908 in cancer. Pencheva et al[23] found that miR-1908 could promote invasion and angiogenesis in melanoma. Xia et al[24] identified that miR-1908 expression was up-regulated in glioblastoma. Overexpression of miR-1908 promoted cell proliferation and invasion through targeting PTEN expression. Recently, Yuan et al[25] found that MicroRNA-1908 is upregulated in human osteosarcoma. Furthermore, the upregulation of miR-1908 can reduce PTEN protein expression in osteosarcoma cells. It is known to all that PTEN repressed cell motility through a variety of pathways. Those results revealed that miRNA-1908 served as an oncogene in osteosarcoma, suggesting that miRNA-1908 could be promising biomarkers for osteosarcoma prognosis.

Conclusions

The expression patterns of miR-1908 in a cohort of patients with osteosarcoma and upregulation of miR-1908 is associated with advanced clinicopathological features and poor prognosis of osteosarcoma. To our knowledge, this is the first time that we identify miR-1908 as a promising biomarker for predicting the prognosis of osteosarcoma.

Conflict of Interest

The Authors declare that they have no conflict of interests.

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