Int J Clin Exp Pathol 2016;9(1):186-190
www.ijcep.com /ISSN:1936-2625/IJCEP0018032

Original Article

Up-regulation of miR-10a and down-regulation of miR-148b serve as potential prognostic biomarkers for osteosarcoma

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Received October 17, 2015; Accepted November 26, 2015; Epub January 1, 2016; Published January 15, 2016

Abstract: Background: MicroRNAs (miRNAs) play key roles in cancer progression. The purpose of this study was to investigate miR-10a and miR-148b expression in osteosarcoma tissues and to further explored the prognostic value of the two miRNAs. Methods: Quantitative real-time PCR method was used to evaluate the expression levels of miR-10a and miR-148b in osteosarcoma (OS) tissues and adjacent non-tumor tissues. Furthermore, we investigated to clarify the relationship of miR-10a and miR-148 with clinicopathological features and survival in OS patients. Results: Our result suggested that miR-10a expression was up-regulated in OS tissues compared to adjacent non-tumor tissues. The expression level of miR-148b was down-regulated in OS tissues compared to adjacent non-tumor tissues. High expressions level of miR-10a and low expressions level of miR-148b were significantly association with advanced TNM stage, and metastasis ($P<0.05$). Furthermore, Kaplan-Meier survival analysis and log-rank test indicated that high expressions of miR-10a and low expression of miR-148b were correlated with shorter overall survival of OS patients (log-rank test, $P<0.05$). Multivariate Cox proportional hazards model showed that increased expression of miR-10a, decreased expression of miR-148b, TNM stage, and metastasis were independent prognostic markers of overall survival of patients. Conclusion: Our data showed that high miR-10a level and low miR-148b level were correlated with aggressive progression and poor prognosis of OS. These miRNAs may have clinical potentials as non-invasive diagnostic biomarkers for OS patients.

Keywords: miR-10a, miR-148b, osteosarcoma, prognosis

Introduction

Osteosarcoma (OS) is one of the most malignant bone tumor in children and adolescents, which is characterized by formation of neoplastic bone tissue [1, 2]. Despite the advances in multiple therapeutic strategies, such as chemotherapy, surgery, and sometimes radiotherapy, overall clinical outcomes for osteosarcoma patients are still dissatisfactory [3, 4]. The 5-year cumulative survival rate for osteosarcoma patients was only 50-60% [5]. Therefore, the discovery of new biomarkers for the diagnosis, prognosis, and treatment of OS remains important.

MicroRNAs (miRNAs) are small non-coding RNAs 18-25 nucleotides in length which regulate gene expression through repressing translation and cleaving their target mRNAs by binding to complementary sites in their 3'-untranslated region (3'-UTR) [6]. Increasing evidence demonstrated that miRNAs were involved in tumor genesis and cancer progression [7]. MiRNAs can function as either tumor suppressors or oncogenes according to their target genes in OS [8]. For example, Liu et al showed that miR-132 inhibited cell growth and metastasis in osteosarcoma cells by downregulation of Sox4 [9]. Jin et al found that miR-539 could suppress osteosarcoma cell invasion and migration by targeting MMP8 [10]. Zhu et al reported that miR-221 promote osteosarcoma cell proliferation, invasion and migration partly through the downregulation of PTEN [11]. Pan et al revealed that miR-27a promoted proliferation, migration and invasion by targeting MAP2K4 in human osteosarcoma cells [12]. These studies suggested that microRNAs can play important roles in OS progression.

Table 1. The relationship of miR-10a and miR-148b expression with clinicopathological features of patients with osteosarcoma

Clinicopathological features	Number	miR-10a expression		miR-148b expression		*P* value (miR-10a)	*P* value (miR-148b)
		Low	High	Low	High		
Age (years)						0.654	0.086
Children + Adolescents	24	11	13	9	15		
Young adults	17	9	8	11	6		
Gender						0.278	0.867
Male	19	11	8	9	10		
Female	22	9	13	11	11		
Tumor size (cm)						0.623	0.262
<5	18	8	10	7	11		
≥5	23	12	11	13	10		
Differentiation						0.279	0.412
Well + moderate	17	10	7	7	10		
Poor	24	10	14	13	11		
TNM stage						0.017	0.005
I + II	15	11	4	3	12		
III + IV	26	9	17	17	9		
Metastasis						0.010	0.002
No	11	9	2	1	10		
Yes	30	11	19	19	11		

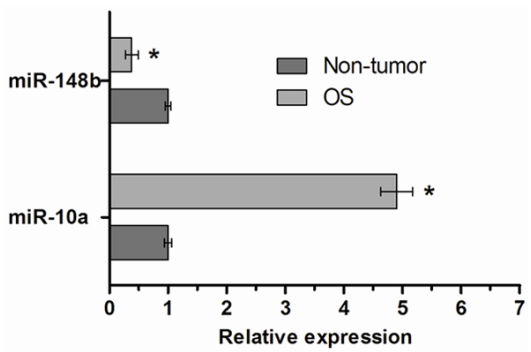

Figure 1. Expression levels of miR-10a and miR-148b in osteosarcoma tissues and adjacent non-tumor tissues.*P<0.05.

In the present study, we examined the expression level of miR-10a and miR-148b in OS tissue samples using quantitative real-time PCR (qRT-PCR). Furthermore, we explored the association of miR-10a and miR-148b level with clinicopathological features and prognosis in OS patients.

Materials and methods

Patients and specimens

A total of 41 samples were collected from patients with osteosarcoma and adjacent non-tumor tissues between 2008 and 2011 from patients who were undergoing surgery in Department of Orthopedics, The First Affiliated Hospital of Xinxiang Medical University. None of the patients enrolled in this study had received chemotherapy or radiotherapy before surgery. All the samples were snap-frozen in liquid nitrogen and then stored at -80°C until use. Moreover, the diagnosis and the histological grading were confirmed by pathologists. The clinicopathological features are summarized in **Table 1**. The present study was approved by the Ethics Committee of the First Affiliated Hospital of Xinxiang Medical University; informed consent was obtained from all of the patients.

Quantitative real-time PCR

Total RNA was extracted using miRNeasy kit (Qiagen) according to the manufacturer's instructions. The expression levels of miRNAs quantitated using the TaqMan miRNA assay kit (Applied Biosystems). Real-time PCR was performed using Rotor Gene 6000 Real-Time PCR (Qiagen) with an Invitrogen kit and a TaqMan universal PCR master mix. The relative expression levels of miRNAs were normalized to that of internal control U6 by using $2^{-\Delta\Delta Ct}$ method.

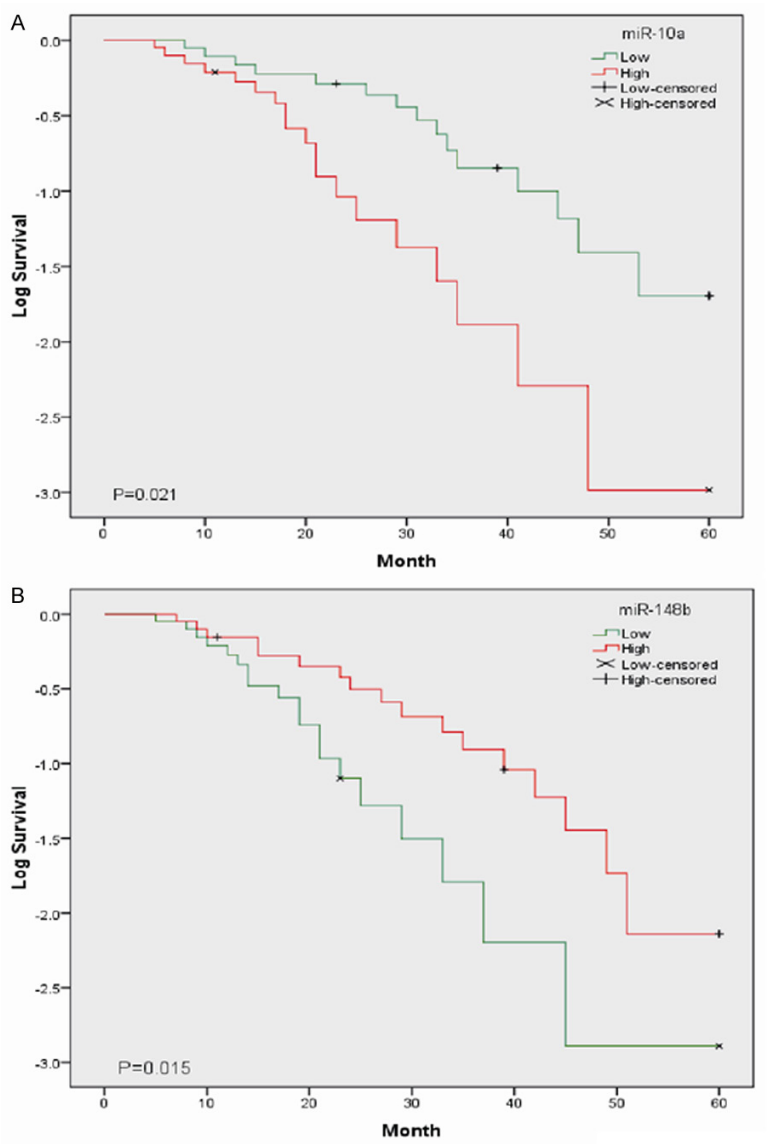

Figure 2. Correlation between miRNAs expression levels and survival in OS patients.

Statistics analyses

All computations were evaluated using SPSS 18.0 version for Windows. Data were expressed as means ± standard deviation (SD). Associations between miRNAs expression level and clinicopathological features were determined using the Chi-square test. Moreover, log-rank test and Kaplan-Meier method were used to evaluate survival rate in patients with osteosarcoma. Multivariate analysis was performed to evaluate prognostic values using Cox proportional hazards model. P<0.05 was considered statistically significant.

Results

We explored the expression level of miR-10a and miR-148b expression levels in OS tissues using quantitative real-time PCR (qRT-PCR). We found that miR-10a expression was significantly increased in OS tissues than that in adjacent non-tumor tissues (P<0.05; **Figure 1**). On the other hand, miR-148b expression was significantly decreased in OS tissues than that in adjacent non-tumor tissues (P<0.05; **Figure 1**).

For evaluation of the correlation between miR-10a/miR-148b expression levels and the clinicopathological features, we categorized the patients into low expression group and high expression group based on the median expression level.

Our data showed that high expression of miR-10a was significantly correlated with advanced TNM stage, and metastasis (P<0.05; **Table 1**). No significant difference was found between miR-10a and age, gender, tumor size, and differentiation (P>0.05; **Table 1**). On the other hand, decreased expression of miR-148b was associated with advanced TNM stage, and metastasis (P<0.05; **Table 1**). Nevertheless, we did not find a significant correlation between miR-148b expression and other clinicopathological features (P>0.05; **Table 1**). In addition, Kaplan-Meier survival and log-rank test analysis showed that the high expressions of miR-10a and low expression of miR-148b were correlated with shorter overall survival (P<0.05; **Figure 2A** and **2B**). Furthermore, Multivariate Cox proportional hazards model analysis revealed that increased expression of miR-10a and decreased expression of miR-148b, advanced TNM stage, and metastasis were independent prognostic fac-

Table 2. Multivariate analysis of the correlation of prognosis miR-10a with clinicopathological features

Clinicopathological features	HR	95% CI	*P* value
Age	0.978	0.612-1.887	0.475
Gender	1.153	0.718-2.704	0.513
Tumor size (cm)	2.035	0.379-5.152	0.091
Differentiation	1.895	0.722-5.912	0.108
TNM stage	2.724	1.638-8.721	0.013
Metastasis	4.106	2.058-10.258	0.002
miR-10a expression	3.237	1.686-9.372	0.005

Table 3. Multivariate analysis of the correlation of prognosis miR-148b with clinicopathological features

Clinicopathological features	HR	95% CI	*P* value
Age	1.106	0.721-2.079	0.385
Gender	0.914	0.685-2.471	0.482
Tumor size (cm)	2.417	0.518-6.074	0.113
Differentiation	2.039	0.812-5.631	0.085
TNM stage	3.014	1.845-9.216	0.008
Metastasis	3.975	1.857-9.824	0.005
miR-148b expression	2.837	1.469-8.165	0.007

tors for overall survival of OS patients (P<0.05; **Tables 2** and **3**).

Discussion

Dysregulation of miRNAs was reported to be associated with the progression of human malignancies [13]. Aberrant expression of miRNAs has been suggested to be as potential biomarkers for diagnosis and prognosis of osteosarcoma [14]. Thus, determination of functional and clinical importance of specific miRNAs may provide effective management of OS. In the present study, the clinical importance of miR-10a and miR-148b in OS were explored and the relationship with clinicopathological features was also determined.

Our data showed that miR-10a was increased in OS tissues compared to the adjacent non-tumor tissues. Moreover, the high expression level of miR-10a was associated with advanced TNM stage, and metastasis. Kaplan-Meier survival and log-rank test analysis showed that high expression of miR-10a was associated with shorter overall survival. Multivariate Cox proportional hazards model analysis indicated that up-regulated expression of miR-10a, advanced TNM stage, and metastasis were independent prognostic factors for overall survival in OS patients. Dysregulation of miR-10a expression has been also reported in kinds of tumors. For example, Safari et al showed that miR-20a expression was significantly up-regulated in cervical cancer and correlated with aggressive progression and poor prognosis of cervical cancer [15]. Yu et al suggested that miR-10a was increased in NSCLC and associated with tumor node metastasis and lymph node metastasis. Furthermore, they found that miR-10a could promote NSCLC cell proliferation, migration and invasion by targeting PTEN [16]. Yan et al suggested that miR-10a was increased in glioma and promoted cell migration and invasion by negatively regulating the expression of EphA8 [17]. However, Khan et al reported that miR-10a expression was significantly decreased in breast cancer and regulated in part through retinoic acid [18]. Our study indicated that miR-10a could act as a tumor oncogene in OS progression.

Furthermore, we found that miR-148b expression was decreased in OS tissues in comparison with adjacent non-tumor tissues and decreased expression of miR-148b was associated with high TNM stage, and metastasis. Furthermore, Kaplan-Meier survival and log-rank test analysis demonstrated that decreased expression of miR-148b was correlated with shorter overall survival. The multivariate Cox proportional hazards model analysis indicated that down-regulated expression of miR-148b, high TNM stage, and metastasis were independent prognostic factors for overall survival in OS patients. Dysregulation expression of miR-148b has been reported in types of tumors. For example, Ghasemkhani et al showed that down-regulated miR-148b expression as predictive biomarker and its prognostic significance associated with clinicopathological features in NSCLC patients [19]. Azizi et al revealed that miR-148b could act as a tumor suppressor genes through suppression of DNA methyltransferase-1 gene in pancreatic cancer cell lines [20]. Zhang et al found that miR-148b could suppress cell proliferation and invasion in hepatocellular carcinoma by targeting WNT1/β-catenin pathway [21]. Our data expanded the tumor suppressive role of miR-148b in OS progression.

In conclusion, these results showed that the expression level of miR-10a and miR-148b contributed to aggressive progression of OS. MiR-10a and miR-148b may serve as non-invasive prognostic biomarkers for OS patients. However, further studies are required to identify the role of these miRNAs in progression and prognosis of osteosarcoma by their targets.

Disclosure of conflict of interest

None.

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