Reduced expression level of *miR-205* predicts poor prognosis in osteosarcoma.

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Abstract

Objective: MicroRNA-205 (*miR-205*), acts as an oncogene or tumor suppressor by modulating the expression of multiple cancer-related target genes. In the present study, we aimed to investigate the clinical significance and prognostic value of *miR-205* in osteosarcoma patients.

Methods: Expression level of *miR-205* was evaluated by qRT-PCR in osteosarcoma tissues and adjacent non-neoplastic tissues. The relationship between the clinicopathological characteristics and *miR-205* was estimated by chi-square test. Associations between overall survival and *miR-205* expression were evaluated using Kaplan-Meier analysis according to log rank test. Cox regression was carried out to determine the prognostic effects of clinicopathological characteristics.

Results: The expression levels of *miR-205* in osteosarcoma tissues were significantly lower than those in noncancerous bone tissues (P<0.001). Low *miR-205* expression was significantly associated with patients with advanced clinical stage (P=0.001) and presence of distant metastasis (P<0.001). We found that the survival time of patients with low *miR-205* expression was shorter than those with high *miR-205* expression (P=0.046). Furthermore, the multivariate analysis with Cox's proportional hazards model confirmed that low *miR-205* expression level was an independent predictor of poor prognosis for the osteosarcoma patients (Hazard ration=2.885; 95% CI: 1.479-8.492, P=0.037).

Conclusions: The present study showed that *miR-205* might be a promising biomarker for the detection of osteosarcoma and its down-regulation might be potentially associated with unfavorable prognosis of osteosarcoma.

Keywords: *miR-205*, Osteosarcoma, Biomarker, Progression, Prognosis.

Introduction

Osteosarcoma, a class of cancer originating from bone, is the most common malignant bone tumor in children and young adults [1-3]. Despite current therapeutic strategies combining adjuvant chemotherapy, surgery and sometimes radiotherapy, the prognosis of osteosarcoma patients remains poor, since about 80% of patients eventually develop recurrent metastatic osteosarcoma following surgical treatment, and the 5-year survival rate of these patients is only 50-60% [4,5]. Thus, new effective drugs and biomarkers to predict its prognosis are needed [6-8]. MicroRNAs (miRNAs) are small RNAs that can regulate gene expression *via* complementary base pairing with the 3' UTR of target mRNAs, resulting in translational repression or mRNA degradation. It has been proposed that miRNAs can play an oncogenic or tumor-suppressive role, depending on the target genes or the cellular context [9,10]. Furthermore, miRNA-expression profiling of human tumours has identified signatures associated with diagnosis, staging, progression, prognosis and response to treatment [11]. MicroRNA-205 (*miR-205*), located in 1q32.2, acts as an oncogene or tumor suppressor by modulating the expression of multiple cancer-related target genes. Previous studies found that the expression of *miR-205* was significantly lower in osteosarcoma tissues than in normal bone tissues, and the abnormal expression of *miR-205* might be a key factor in osteosarcoma progression [12-14]. In the present study, we aimed to investigate the clinical significance and prognostic value of *miR-205* in osteosarcoma patients.

Materials and Methods

Patient samples

A total of 103 pairs of osteosarcoma tissues and their matched adjacent normal bone tissues were obtained from patients who

underwent surgery at the Qilu Hospital of Shandong University between 2010 and 2016. All patients enrolled in this study offered written informed consent. This study was approved by the Ethical and Scientific Committees of the Qilu Hospital of Shandong University, and it was performed in compliance with the Declaration of Helsinki Principles. All tissue specimens obtained from patients with osteosarcomas were handled and made anonymous according to the ethical and legal standards. All the patients recruited into the present study did not receive radiotherapy or chemotherapy or any other treatment before and after operation. At the time of surgery, all tissue samples were immediately flash frozen in liquid nitrogen and stored at -80°C until use. The diagnosis of osteosarcoma was confirmed pathologically. The clinicopathological characteristics of osteosarcoma patients are summarized in Table 1.

Quantitative real-time PCR

Total RNAs were extracted from the tissues using Trizol reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions. cDNA was reverse transcribed from total RNA samples using specific *miR-205* primers from the TaqMan MicroRNA Assays and reagents from the TaqMan MicroRNA Reverse Transcription kit (Applied Biosystems, Foster City, CA, USA). Quantitative reverse-transcription polymerase chain reaction (RT-qPCR) assays were performed to evaluate *miR-205* expression in clinical osteosarcoma tissues and non-neoplastic bone tissues. The concentration and purity of all RNA samples were detected by NanoDrop ND-2000 spectrophotometer (NanoDrop Technologies, Houston, TX, USA). NCodeTM SYBR$^{®}$ Green miRNA qRT-PCR Kit (Invitrogen, Carlsbad, CA, USA) was used to synthesize specific cDNA of *miR-205* and U6 (as an internal control), and perform qRT-PCR, which was analysed with the DNA Engine Opticon 2 Real-Time Cycler (MJ Research Inc., Waltham, MA, USA) according to the manufacturer's instructions. Each sample was examined in triplicate and the $2^{-\Delta\Delta Ct}$ method was used to quantify the relative amount of *miR-205*.

Statistical analysis

The t-test was used to compare differences in miRNA expression between osteosarcoma tissues and noncancerous bone tissues. The relationship between the clinicopathological characteristics and *miR-205* was estimated by chi-square test. Associations between overall survival and *miR-205* expression were evaluated using Kaplan-Meier analysis according to log rank test. Cox regression was carried out to determine the prognostic effects of clinicopathological characteristics. A value of P<0.05 was considered to indicate a statistically significant result. All analyses were performed using SPSS version 18.0 (Statistical Package for the Social Sciences, Chicago, IL, USA).

Results

Expression of miR-205 is down-regulated in osteosarcoma tissues

miR-205 expression levels in osteosarcoma tissues and adjacent normal bone tissues were detected by qRT-PCR. We found that the expression levels of *miR-205* in osteosarcoma tissues were significantly lower than those in noncancerous bone tissues (P<0.001, Figure 1). To investigate the associations of *miR-205* expression with the clinicopathological features of osteosarcoma patients, we divided the 103 patients into two groups using the median value of *miR-205* expression levels in osteosarcoma patients as a cut-off point. Of 103 osteosarcoma specimens, 51 highly expressed *miR-205*, and 52 lowly expressed *miR-205*.
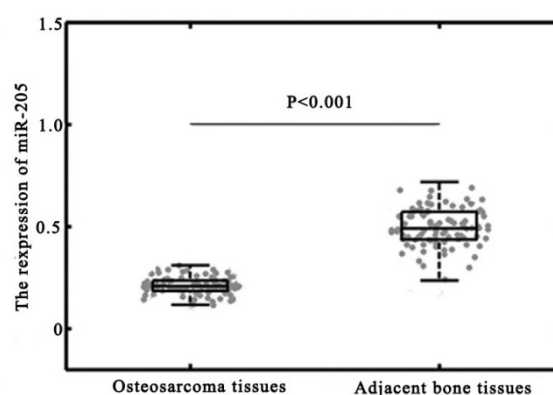

Figure 1. miR-205 expression in osteosarcoma tissues and non-neoplastic bone tissues was detected by qRT-PCR assay.

Relationship between miR-205 expression in osteosarcoma patients and clinicopathological features

We compared the clinicopathological factors of the high and low *miR-205* expression group and found that low *miR-205* expression was significantly associated with patients with advanced clinical stage (P=0.001) and presence of distant metastasis (P<0.001). However, none associations between *miR-205* expression and other clinical characteristics were observed, including age, gender, tumor size, and anatomic location (all P>0.05, Table 1).

Expression level of miR-205 predicts prognosis of patients with osteosarcoma

To further evaluate whether *miR-205* expression levels were associated with prognosis of patients with osteosarcoma, we performed survival analysis. As showed in Figure 2, the survival time of patients with low *miR-205* expression was shorter than those with high *miR-205* expression (P=0.046). Log-rank test found that high *miR-205* expression (both P<0.001), large tumor size (both P=0.03), advanced clinical stage (P=0.006 and 0.01, respectively), presence of distant

metastasis (P=0.003 and 0.008, respectively), and poor response to chemotherapy (both P=0.02) were significantly associated with overall and disease-free survivals of osteosarcoma patients (Table 2). Furthermore, the multivariate analysis with Cox's proportional hazards model confirmed that low *miR-205* expression level was an independent predictor of poor prognosis for the osteosarcoma patients (Hazard ration=2.885; 95% CI: 1.479-8.492, P=0.037, Table 3).
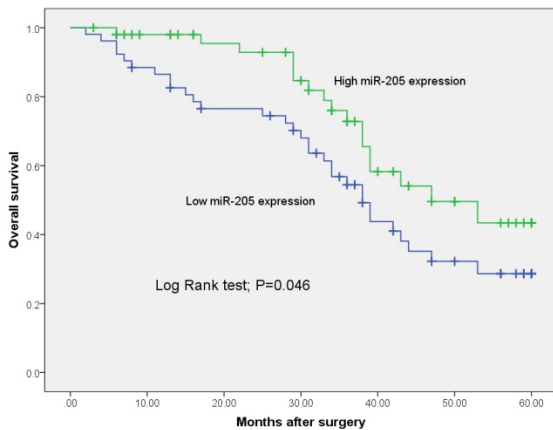

Figure 2. Kaplan-Meier curves for survival time in patients with osteosarcoma divided according to miR-205 expression.

Table 1. miR-205 expression level with clinicopathological features of osteosarcoma.

Variables	Case (n)	miR-205 expression level		P value
		Low (n=52)	High (n=51)	
Age				
≤ 45	59	27	32	0.321
>45	44	25	19	
Gender				
Male	61	29	32	0.549
Female	42	23	19	
Tumor size (cm)				
≤ 8	57	26	31	0.324
>8	46	26	20	
Anatomic location				
Tibia/femur	79	39	40	0.816
Elsewhere	24	13	11	
=Stage				
IIA	39	11	28	0.001
IIB/III	64	41	23	
Distant metastasis				
Absent	82	33	49	<0.001
Present	21	19	2	

Table 2. Univariate survival analyses of overall and disease-free survival in 103 patients with osteosarcoma.

Variable	Overall survival			Disease-free survival		
	Hazard ratio	95% CI	P-value	Hazard ratio	95% CI	P-value
Age	1.43	0.34-2.97	NS	3.92	1.27-9.01	NS
Gender	0.52	0.28-1.18	NS	0.43	0.07-1.27	NS
Tumor size	2.13	0.72-4.35	0.03	1.79	0.81-3.92	0.03
Anatomic location	1.18	0.16-4.72	NS	1.63	0.96-3.99	NS
Serum level of lactate dehydrogenase	2.19	0.89-4.17	NS	1.17	0.91-3.18	NS
Serum level of alkaline phosphatase	1.62	0.64-4.88	NS	1.91	0.88-3.07	NS
Clinical stage	3.45	1.08-8.23	0.006	3.09	0.68-7.38	0.01
Distant metastasis status	3.87	1.28-9.32	0.003	3.18	1.47-8.38	0.008
Response to chemotherapy	2.89	1.02-6.78	0.02	2.63	0.97-4.98	0.02
miR-203 expression	2.73	1.69-8.91	0.01	4.19	2.91-10.12	0.005

NS: refers to differences without statistical significance.

Table 3. Multivariate analyses of prognostic parameters in patients with osteosarcoma by Cox regression analysis.

Variable	Hazard ratio	95% CI	P-value
Age	1.729	0.683-2.982	0.127
Gender	0.732	0.381-2.018	0.893
Tumor size	2.881	0.799-6.012	0.091
Anatomic location	1.562	0.283-2.181	0.782
Stage	3.198	2.117-10.283	<0.001

Distant metastasis	4.293	2.378-16.338	<0.001
miR-205 expression level	2.885	1.479-8.492	0.037

Discussion

Osteosarcoma is the most common malignant bone tumor in children and adolescents [4]. Twenty percent of patients present with clinically detectable metastases, with micrometastases presumed to be present in many of the remaining patients. Despite the aggressive use of surgery, chemotherapy, and/or radiotherapy, the survival for osteosarcoma patients remains poor. Thus, studies to address genes involved in osteosarcoma carcinogenesis may help understand the complex molecular mechanisms underlying osteosarcoma progression and providing potential therapeutic targets and prognostic biomarkers for the clinical treatment [5].

In recent years, microRNA expression profiling has indeed provided evidence of the association of these tiny molecules with tumor development and progression. An increasing number of studies have then demonstrated that microRNAs can function as potential oncogenes or oncosuppressor genes, depending on the cellular context and on the target genes they regulate [15,16]. Therefore, miRNAs play an important role in tumorigenesis and metastasis.

miR-205 conducts conflicting functions in human cancers, where it may function either as a tumor suppressor or an oncogene in a single tumor type. For example, *miR-205* was found to be significantly overexpressed in human cervical cancer tissues and promotes proliferation and migration of cervical cancer cells by targeting CYR61 and CTGF [17]. Furthermore, serum *miR-205* expression level was significantly upregulated in cervical cancer patients compared with healthy donors, and Kaplan-Meier survival analysis showed that cervical cancer patients with high *miR-205* expression tended to have shorter overall survival, indicating that *miR-205* might be a predictive biomarker for the prognosis of cervical cancer patients [18]. Hou et al. found that *miR-205* expression was significantly lower in glioma tissues than in non-neoplastic brain tissues. Survival analysis demonstrated that the cumulative 5 y overall survival rate of patients with glioma in the high *miR-205* expression group was significantly higher than that in the low *miR-205* expression group, and multivariate Cox regression analysis further indicated that *miR-205* expression was an independent prognostic indicator of the overall survival of patients with glioma, suggesting that *miR-205* holds potential as a prognostic factor for glioma [19]. Furthermore, serum *miR-205* expression was found to be a novel and valuable biomarker for the diagnosis of glioma and a prognostic factor for those with a tumor at an advanced pathological grade [20]. In the study by Orang et al. their data suggested that *miR-205* could be reduced in the majority of metastatic Colorectal Cancer (CRC) and the risk of CRC metastasis may be predicted by monitoring *miR-205* in CRC samples collected at the time of the initial diagnosis. In addition, *miR-205* is capable of distinguishing lymph node metastatic CRC from nonmetastatic CRC [21].

In previous studies, *miR-205* was found to be acted as a tumor suppressor by modulating the expression of multiple cancer-related target genes. Yang et al. found that the expression of *miR-205* was significantly lower in osteosarcoma tissues than in normal bone tissues, and *miR-205* inhibited cell proliferation, invasion, and migration *via* targeting TGF-α. Therefore, *miR-205* may function as a tumor suppressor *via* targeting TGF-α in osteosarcoma, and the abnormal expression of *miR-205* might be a key factor in osteosarcoma progression [14]. Furthermore, Wang et al. found that *miR-205* suppressed the proliferative and migratory capacity of human osteosarcoma Mg-63 cells by targeting VEGFA [13]. Zhang et al. found that *miR-205* acted as a tumor suppressor in osteosarcoma *via* directly targeting RUNX2 [12]. However, the clinical significance and prognostic value of *miR-205* in osteosarcoma have not been investigated. Therefore, we aimed to investigate the clinical significance and prognostic value of *miR-205* in osteosarcoma patients.

In the present study, we found that the expression levels of *miR-205* in osteosarcoma tissues were significantly lower than those in noncancerous bone tissues. We then compared the clinicopathological factors of the high and low *miR-205* expression group and found that low *miR-205* expression was significantly associated with patients with advanced clinical stage and presence of distant metastasis. To further evaluate whether *miR-205* expression levels were associated with prognosis of patients with osteosarcoma, we performed survival analysis. We found that the survival time of patients with low *miR-205* expression was shorter than those with high *miR-205* expression. Furthermore, the multivariate analysis with Cox's proportional hazards model confirmed that low *miR-205* expression level was an independent predictor of poor prognosis for the osteosarcoma patients.

In conclusion, the present study showed that *miR-205* may be a promising biomarker for the detection of osteosarcoma and its down-regulation may be potentially associated with unfavorable prognosis of osteosarcoma, suggesting that *miR-205* might serve as a potential biological marker for further risk stratification of osteosarcoma.

Conflicts of Interest

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