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miR-497 as a potential serum biomarker for the diagnosis and prognosis of osteosarcoma

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Abstract. – OBJECTIVE: The aim of this manuscript is to analyze the diagnostic and prognostic value of circulating miR-497 in the plasma of patients with osteosarcoma.

PATIENTS AND METHODS: Serum miR-497 expression levels were measured by quantitative real-time reverse transcription-polymerase chain reaction (qRT-PCR). Correlations between miR-497 expression and the clinicopathological features and prognosis of osteosarcoma patients were then evaluated. The receiver operating characteristic curve (ROC) and the area under the curve (AUC) were used to evaluate diagnostic accuracy.

RESULTS: Our data showed that serum miR-497 expression was down-regulated in osteosarcoma patients compared with the matched healthy controls ($p < 0.001$). Then, low miR-497 expression was significantly associated with clinical stage ($p = 0.001$), distant metastasis ($p = 0.001$) and response to chemotherapy ($p = 0.007$). The receiver operating characteristic (ROC) curve analysis of the accuracy in distinguishing osteosarcoma patients from healthy controls yielded an area under the curve (AUC) value of 0.848 (95% confidence interval (CI), 0.773-0.923). Kaplan-Meier analysis results showed that patients with lower expression of miR-497 had shorter survival times ($p < 0.001$). Univariate and multivariate analyses revealed that the miR-497 expression level and various clinicopathological features were independent prognostic parameters.

CONCLUSIONS: Our data showed that low serum miR-497 level was correlated with aggressive progression and poor prognosis of osteosarcoma. Serum miR-497 may be a potential biomarker for early detection and clinical evaluation in patients with osteosarcoma.

Key Words:
miR-497, Serum, Diagnosis, Prognosis, Osteosarcoma.

Introduction

Osteosarcoma (OS) accounts for approximately 20% of all primary bone cancers and is the second highest cause of cancer-related death in the pediatric age group[1,2]. Despite the rapid development of therapeutic strategies, such as wide tumor excision, and radiotherapy, long-term survival rate of patients diagnosed with advanced OS remains very low[3,4]. Early diagnosis and prognostic evaluation of OS are crucial for timely and appropriate treatment. Thus, a novel biomarker for OS diagnosis and prognosis is of vital importance.

miRNAs, small non-coding RNA gene products of approximately 22 nucleotides, have been shown to play an important role in regulating the growth of many[5-7]. Emerging evidence showed that miRNAs participated in human tumorigenesis and/or metastasis targeting directly oncogenes or tumor suppressor genes[8,9]. The expression profiling of miRNAs has already entered into cancer clinics as diagnostic and prognostic biomarkers[10]. Up-regulation of miR-497 has been reported to inhibit cell proliferation, migration, and invasion by targeting AMOT, suggesting its potential as a target for the treatment of this malignancy[11]. However, the role of miR-497 expression in diagnosis and prognosis of OS has been well less elaborated. Therefore, in the present study, we focus on miR-497.

Patients and Methods

Patients

Between June 2008 and February 2014, 185 patients of newly diagnosed OS from The Affiliated Hospital of Qingdao University, were recruited in this study. Following the diagnosis, all 108 patients with OS were treated with the same neoadjuvant chemotherapy. Preoperative plasma samples were collected from 185 OS patients as well as 130 healthy volunteers and then frozen and stored at -80°C for RNA extraction. The detailed clinical data including Age (year), Gender,

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Tumor size (cm), Anatomic location, Serum level of lactate dehydrogenase, serum level of alkaline phosphatase, clinical stage, distant metastasis, and response to chemotherapy were collected and stored in a database. The follow-up information of all participants was updated every 3 months by telephone. Our study protocol was recognized by Research Ethics Committee in The Affiliated Hospital of Qingdao University. Written informed consent was obtained from each participant involved in advance.

Quantitative Real-time Polymerase Chain Reaction (qRT-PCR)

RNA extraction was performed from fresh serum following the instructions of a miRcute miRNA isolation kit (Invitrogen, San Diego, CA, USA). The reverse transcription was conducted using a TaqMan MicroRNA Reverse Transcription Kit (MBI Fermentas, Burlington, ON, Canada) according to the manufacturer's protocol. Real-time polymerase chain reaction (PCR) was carried out using an Applied Biosystems StepOnePlus Sequence Detection System (Invitrogen, Foster City, CA, USA). The small nuclear RNA U6 was selected as an internal control. Data analysis was done by the CT method for relative quantification. For each sample, all experiments were done in triplicate.

Statistical Analysis

All data are presented as mean ± SD. Statistical significance of the data was using the Statistical Package of SPSS version 13.0 for Windows (SPSS Inc., Chicago, IL, USA). A chi-square test was used to analyze the relationship between miR-497 and various clinicopathologic parameters. The receiver operating characteristic (ROC) curves were established for discriminating patients with OS from the normal controls. Overall survival curves were estimated by the Kaplan-Meier method. The Cox proportional hazards model was used in the multivariate analysis. Two-tailed *p*-values less than 0.05 were considered statistically significant.

Results

miR-497 Down-regulation in Human OS

Serum miR-497 expression was detected in 185 pairs of OS patients and the matched healthy controls by qRT-PCR. The results showed that the miR-497 expression level in the serum from OS patients was significantly lower than that from controls (Figure 1, $p < 0.01$).

Association Between Clinicopathological Characteristics and miR-497 Expression in OS Patients

We further analyzed the association between the expression of miR-497 and clinicopathological characteristics of OS. According to the expression of miR-497, these cases were divided into a high miR-497 expression group (n = 94) and a low expression group (n = 91), based on the median value of serum miR-497 expression levels in OS patients as a cutoff point (4.80). As summarized in Table I, low miR-497 expression was significantly associated with clinical stage ($p = 0.001$), distant metastasis ($p = 0.001$) and response to chemotherapy ($p = 0.007$). However, No significant differences were observed between serum miR-497 expression and other parameters.

Diagnosis Value of Serum miR-497 for OS

The ROC curve analysis was performed to evaluate the value of plasma miR-497 in discriminating between OS patients and matched normal controls. The results showed that plasma miR-497 could function as valuable biomarkers for OS cases from healthy controls with an AUC of 0.848 (95% CI: 0.773-0.923; $p < 0.001$, Figure 2). The cut-off value was 0.995 with the highest

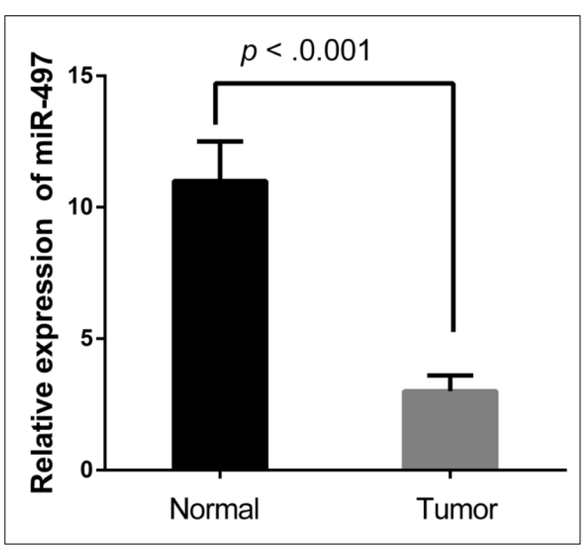

Figure 1. Comparison of miR-497 expression between OS patients and the matched healthy controls.

Table I. Association of serum miR-497 expression with clinicopathological features of osteosarcoma.

Characteristics	n	High expression	Low expression	p
Age (year)				0.382
< 55	73	40	33	
≥ 55	112	54	58	
Gender				0.219
Female	75	34	41	
Male	110	60	50	
Tumor size (cm)				0.593
≥ 8	85	45	40	
< 8	100	49	51	
Anatomic location				0.637
Tibia/femur	119	62	57	
Elsewhere	66	32	34	
Serum level of lactate dehydrogenase				0.489
Elevated	103	50	53	
Normal	82	44	38	
Serum level of alkaline phosphatase				0.530
Elevated	114	60	54	
Normal	71	34	37	
Clinical stage				0.001
IIA	111	74	37	
IIB/III	74	20	54	
Distant metastasis				0.001
Absent	128	80	48	
Present	57	14	43	
Response to chemotherapy				0.007
Good	98	59	39	
Poor	87	35	52	

specificity and sensitivity. Taken together, these data showed that decreased serum miR-497 level is a potential biomarker for the diagnosis of OS.

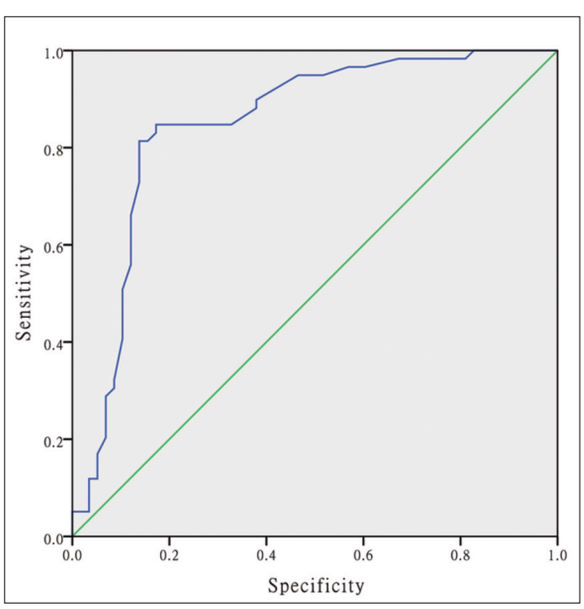

Figure 2. ROC-Curve for serum miR-497 as a diagnostic marker for OS.

Association of miR-497 Expression with Clinical Prognosis

Kaplan-Meier survival curves of correlation between survival and plasma miR-497 levels are shown in Figure 3. The low miR-497 expression patients survived shorter than those OS patients with high miR-497 expression ($p < 0.001$). Univariate analysis indicated that clinical stage, distant metastasis status, response to chemotherapy, and miR-497 expression were statistically significant risk factors affecting the overall survival of patients with OS (Table II, All $p < 0.05$). Multivariate analysis confirmed that miR-497 expression level ($p = 0.013$) was independently associated with the OS (Table II).

Discussion

Up to date, emerging evidence demonstrated that several tumor-specific features could be detected in human serum or plasma samples and be widely used in diagnosing serious diseases[12,13]. Over the past 10 years, accumulating evidence strongly informed that miRNAs play an impor-

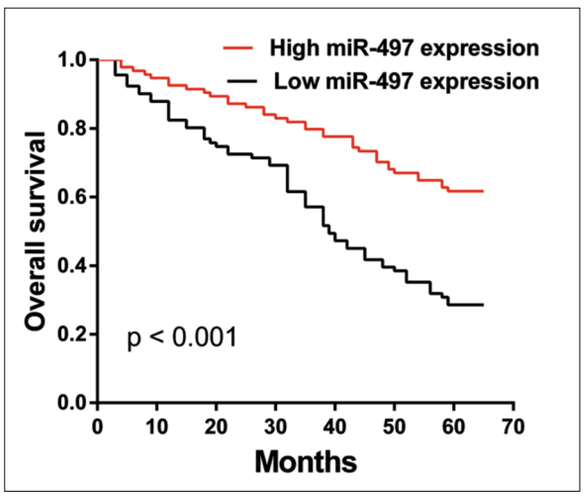

Figure 3. Kaplan-Meier curves for survival time in patients with OS divided according to miR-497 expression levels.

tant role in crucial cellular processes of cancer development[14], miRNAs in serum/plasma are emerging as a new class of potential markers for noninvasive diagnosis and monitoring of cancer[15]. Therefore, much effort has been made to screening reliable biomarkers in serum miRNAs for the diagnosis and prognosis evaluation of OS.

MiR-497, a highly conserved miRNA encoded by the first intron of the MIR497HG gene on human chromosome 17p13.1[16]. Previous studies have demonstrated that miR-497 exerts a tumor suppressor function in human cancer. For example, Furuta et al[17] reported that miR-497 suppressed cell growth by targeting multiple cell-cycle regulators in HCC. Qiu et al[18] found that microRNA-497 inhibits invasion and metastasis of colorectal cancer cells by targeting vascular endothelial growth factor-A. Wu et al[19] showed that miR-497 suppresses angiogenesis in breast carcinoma by targeting HIF-1. Du et al[20] identified miR-497 as tumor suppressor which could be a potential diagnostic marker for bladder cancer. Ruan et al[10] observed that miR-497 inhibits cell proliferation, migration, and invasion by targeting AMOT in human OS cells. Shao et al[21] found that the down-regulation of microRNA-497 contributes to cell growth through PI3K/Akt Pathway in OS. Those results revealed that miR-497 could be promising novel circulating biomarkers in clinical detection of OS.

In the present study, we firstly showed that the expression of miR-497 was significantly lower in the serum of OS patients. Then, we found that low miR-497 expression was significantly associated with clinical stage, distant metastasis and response to chemotherapy. ROC analyses demonstrated that miR-497 had significantly higher sensitivity and specificity in distinguishing the individuals with OC from other groups of individuals. Furthermore, Kaplan-Meier survival analysis showed that patients with low miR-497 expression had significantly poor overall survival rate. At last, multivariate analyses demonstrated that low miR-497 expression was an independent prognostic factor. These findings suggested that serum miR-497 could be a potential prognostic and diagnostic biomarker for OS patients.

Conclusions

miR-497 was significantly downregulated in OS serum. It could serve as promising biomarker for both diagnosis and prognosis of OS. The biological functions of miR-497 and its role as a tumor suppressor are worth to be further investigated.

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Table II. Univariate and multivariate analyses for overall survival by Cox regression model.

Variables	Univariate analysis			Multivariate analysis		
	HR	95% CI	p	HR	95% CI	p
Serum miR-497 expression	4.127	2.371-9.673	0.006	3.785	1.993-8.574	0.004
Clinical stage	2.361	1.792-7.844	0.009	2.045	1.454-6.645	0.003
Distant metastasis status	3.938	2.249-8.659	0.013	3.159	2.044-8.229	0.008
Response to chemotherapy	2.459	1.881-6.773	0.018	2.095	1.769-6.318	0.013

Conflict of Interest

The Authors declare that there are no conflicts of interest.

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