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Decreased expression of miR-490-3p in osteosarcoma and its clinical significance

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Abstract. – OBJECTIVE: Increasing evidence has revealed that miRNAs play a key role in the development and progression of tumor and are being explored as a prognostic biomarker. The aim of this study was to investigate the clinical significance of miR-490-3p expression in osteosarcoma.

PATIENTS AND METHODS: Quantitative real-time PCR (qRT-PCR) was used to evaluate miR-490-3p level in osteosarcoma tissues. We also analyzed the correlations between the expression level of miR-490-3p and the clinical characteristics in cases of osteosarcoma. Also, Kaplan-Meier curve and the log-rank test were conducted to detect the prognostic value of miR-490-3p. Finally, univariable and multivariable Cox regression analyses were used to evaluate independent prognostic factors.

RESULTS: MiR-490-3p expression level was significantly downregulated in osteosarcoma tissues in comparison with noncancerous bone tissues. Moreover, low miR-490-3p expression was significantly associated with distant metastasis, advanced clinical stage and poor overall survival and relapse-free survival. Univariate and multivariate analysis results indicated that miR-490-3p was an independent prognostic factor in osteosarcoma.

CONCLUSIONS: Our results showed that miR-490-3p plays critical roles in osteosarcoma progression and serves as a novel prognostic indicator and a potential therapeutic target for osteosarcoma.

Key Words:
miR-490-3p, Prognosis, Overall survival, Relapse-free survival, Osteosarcoma.

Introduction

Osteosarcoma is one of the most common primary bone malignancy that commonly occurs with a high rate in teenagers and often leads to mortality[1,2]. Although treatment strategies have been improved over the past four decades, little has changed for patients with metastatic disease, and their long-term survival rate remained at 25-30%[3]. Although recent developments in molecular biology have provided insight into the molecular pathogenesis of osteosarcoma, the molecular mechanisms are still elusive[4]. Therefore, there is a great need to explore novel and highly sensitive molecular biomarkers with reliable clinical significance to improve clinical outcome of patients suffering osteosarcoma.

MicroRNAs (miRNAs) represent a class of endogenous, highly conserved, small nonprotein-coding RNAs that are approximately 22 nucleotides in length[5,6]. miRNAs appear to play a role in carcinogenesis by modulating the expression of tumor suppressor genes and oncogenes to generate a complex combinatorial network[7-9]. miR-490-3p has been reported to serve as a tumor suppressor in several tumors, including breast cancer[10], endometrial carcinoma[11], and colorectal cancer[12]. A previous study[13] showed that miR-490-3p was down-regulated in osteosarcoma cell lines. However, to our best knowledge, the potential role of miR-490-3p as a prognostic biomarker in osteosarcoma has not been investigated.

In the present work, we detected the expression levels of miR-490-3p in osteosarcoma patients to determine whether there was a correlation between its expression and the clinical outcomes of osteosarcoma patients. Our results showed that miR-490-3p was remarkably down-regulated in osteosarcoma and could be served as a potential prognostic biomarker for patients.

Patients and Methods

Patients and Tissue Samples

148 paired osteosarcoma tissues and adjacent non-tumor tissues used in this study were collected from patients who underwent surgical resec-

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tion at Tai'an City Central Hospital. Surgically removed tissues were quickly frozen in liquid nitrogen until analysis. Eligibility included histologically confirmed osteosarcoma, no distant metastases before surgery, and no preoperative chemotherapy or locoregional therapy. Demographic information of those patients and clinical features was collected. The clinicopathological features of the patients were summarized in Table I. Tai'an City Central Hospital Ethical Committee approved this study, and informed consent was given for the use of clinical specimens in this study.

RNA Isolation and Quantitative Real-time PCR

Total RNA from cells and tissues was isolated using TRIzol reagent (Invitrogen, Carlsbad, CA, USA) and was eluted in 50 μL nuclease free water. The isolated RNA was quantified by measuring its absorbance at 260 nm. Real-time PCR was performed using SYBR Premix Ex Taq (TaKaRa) and measured in a LightCycler 480 system (Roche, Basel, Switzerland). U6 was used as internal control. The forward primers used for miR-490-3p amplification were shown in Table II. The ΔΔCt method was used for relative quantification, and the miR- 490-3p level was expressed as a relative value to the control group.

Statistical Analysis

All results are presented as the mean values ± SEM. Student's *t*-test and the chi-square test were used to determine the differences among different groups. Overall survival was defined as the interval from the date of surgery to osteosarcoma-related death. Kaplan-Meier survival curves were used to estimate the relapse-free survival and overall survival of the patients which was determined using the log-rank test. The survival data were evaluated using univariate and multivariate Cox regression analyses. $p < 0.05$ was considered statistically significant. The SPSS 18.0 software package (SPSS Inc., Chicago, IL, USA) was used for statistical analysis.

Table I. Correlation of miR-490-3p expression with clinicopathological features of osteosarcoma.

Clinicopathological features	Number of cases	miR-490-3p expression		*p*-value
		High	Low	
Age				0.443
< 55 years	57	30	27	
≥ 55 years	91	42	49	
Gender				0.760
Male	101	50	51	
Female	47	22	25	
Tumor size				0.471
≥ 8 cm	99	48	51	
< 8 cm	59	24	25	
Anatomic location				0.907
Tibia/femur	85	41	44	
Elsewhere	63	31	32	
Serum level of lactate dehydrogenase				0.147
Elevated	107	56	51	
Normal	41	16	25	
Serum level of alkaline phosphatase				0.986
Elevated	78	48	40	
Normal	70	34	36	
Clinical stage				0.000
IIA	81	50	31	
IIB/III	67	22	45	
Distant metastasis				0.005
Absent	78	45	33	
Present	70	27	43	
Response to chemotherapy				0.829
Good	63	30	33	
Poor	85	42	43	

Table II. Primer sequences.

Real-time quantitative RT-PCR primers	Sequences
miR-490-3p (forward)	5'-GCAAACAACCAUUCGGCUGUC-3'
miR-490-3p (reverse)	5'-CGCAGGTCCGGAGTAGGT-3'
U6 (forward)	5'-CTCGCTTCGGCAGCACA-3'
U6 (reverse)	5'-AACGCTTCACGAATTTGCGT-3'

Results

miR-490-3p is Downregulated in Osteosarcoma Tissues

The miR-490-3p levels in the 148 pairs of resected specimens (tumor tissue samples and matched adjacent non-tumor tissue samples) from osteosarcoma patients were evaluated by qRT-PCR. Our results showed that miR-490-3p levels were down-regulated in osteosarcoma (Figure 1, $p < 0.01$), which might imply the involvement of miR-490-3p in osteosarcoma.

Correlations Between miR-490-3p Expression and Clinicopathologic Features of Osteosarcoma Patients

To further understand the significance of miR-490-3p expression in osteosarcoma patients, patients were stratified into two groups based on the dichotomized scores. Table I showed the clinicopathological differences between high and low miR-490-3p expression groups. Increased miR-490-3p expression in osteosarcoma was found to be significantly associated with clinical stage ($p = 0.000$) and distant metastasis ($p < 0.005$). No significant difference was observed between miR-490-3p expression and patients' age, gender, tumor size, anatomic location, serum level of alkaline phosphatase, response to chemotherapy.

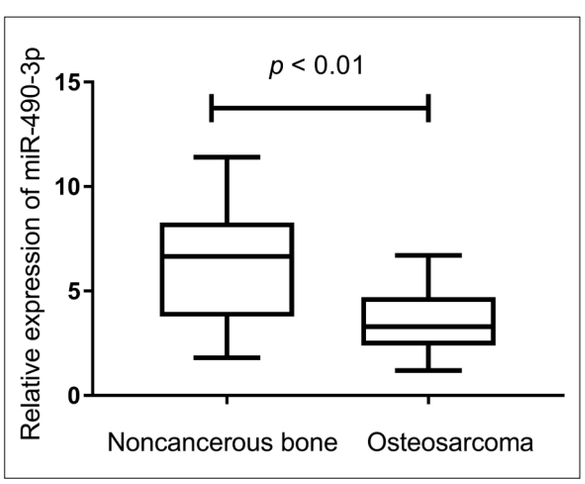

Figure 1. miR-490-3p expressions in osteosarcoma and corresponding noncancerous bone tissues were respectively detected by RT-PCR.

miR-490-3p Serves as an Independent Prognostic Predicator for Patients with Osteosarcoma

To evaluate the prognostic value of the miR-490-3p expression in osteosarcoma, survival curves were constructed by Kaplan-Meier method and compared by the log-rank test. As shown in Figure 2, our results revealed that the patients in the low miR-490-3p expression group had poorer overall survival rate ($p < 0.001$) and worse relapse-free survival rate ($p = 0.001$). Moreover, the univariate analysis identified clinical stage, distant metastasis and low expression of miR-490-3p as prognostic factors, whereas age, gender, tumor size, serum level of alkaline phosphatase and response to chemotherapy were not significantly associated with overall survival (Table III). Multivariate Cox regression analysis identified miR-490-3p an independent prognostic factor in patients with osteosarcoma (Table IV, $p = 0.003$).

Discussion

It has been widely accepted that miRNAs are involved in multiple cellular functions such as differentiation, proliferation, and apoptosis. An increasing number of studies showed that miRNAs functioned as either tumor suppressors or oncogenes by repressing the expression of important cancer-related genes[14]. For example, Li et al[15] showed that miR-143 could promote apoptosis of osteosarcoma cells by caspase-3 activation via targeting Bcl-2. Liu et al[16] found that miR-335 suppressed osteosarcoma cell proliferation by targeting surviving. Zhang et al[17] demonstrated that miR-198 acts as a tumor suppressor in osteosarcoma by down-regulation of ROCK1.

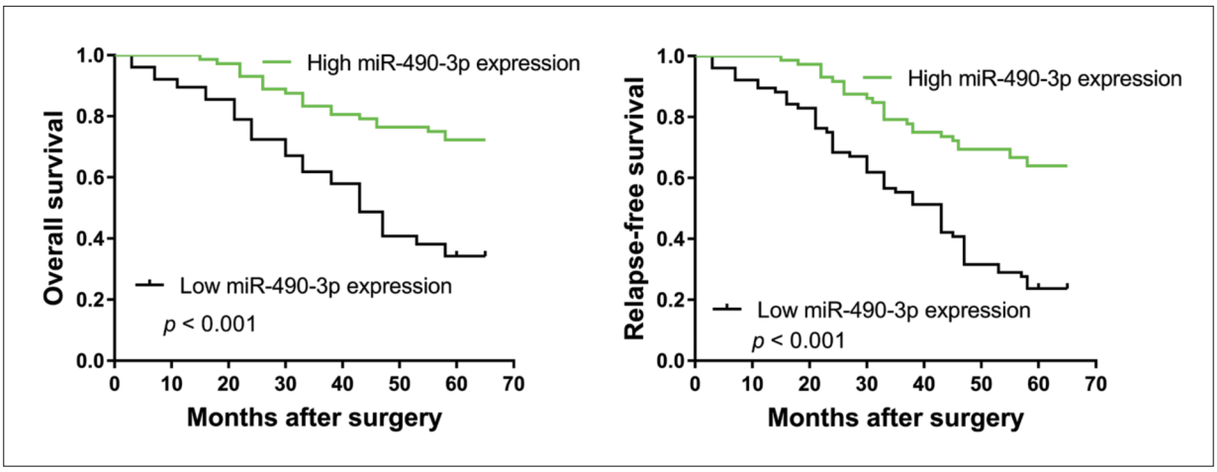

Figure 2. Kaplan-Meier curves for survival time in patients with osteosarcoma divided according to miR-490-3p expression. ***A,*** Patients with lower miR-490-3p expression level are associated with a poorer OS. ***B,*** Patients with lower miR-490-3p expression level are associated with a poorer RFS.

Table III. Univariate analysis of prognostic factors in patients with osteosarcoma.

Variables	Risk ratio	95% CI	*p*-value
Age			
< 55 *vs.* ≥ 55	0.623	0.214-1.554	0.316
Gender			
Male *vs.* Female	1.673	0.662-3.317	0.247
Tumor size			
≥ 8 *vs.* < 8	1.468	0.732-2.993	0.184
Anatomic location			
Tibia/femur *vs.* Elsewhere	1.021	0.335-0.741	0.734
Serum level of lactate dehydrogenase			
Elevated *vs.* Normal	0.773	0.258-2.137	0.451
Serum level of alkaline phosphatase			
Elevated *vs.* Normal	0.977	0.456-2.148	0.266
Clinical stage			
IIA *vs.* IIB/III	4.459	2.371-9.663	0.002
Distant metastasis			
Absent *vs.* Present	3.761	1.532-6.591	< 0.001
Response to chemotherapy			
Good *vs.* Good	3.137	1.764-4.033	0.219
miR-490-3p expression			
High *vs.* Low	2.134	1.328-7.762	< 0.001

Table IV. Multivariate analysis of prognostic factors in patients with osteosarcoma.

Variables	Risk ratio	95% CI	*p*-value
Clinical stage			
IIA *vs.* IIB/III	3.893	1.833-7.562	0.007
Distant metastasis			
Absent *vs.* Present	2.639	1.438-5.238	0.006
miR-490-3p expression			
High *vs.* Low	1.833	1.014-6.833	0.003

Meanwhile, some miRNAs function as oncogene in osteosarcoma development. Yuan et al[18] reported miR-1908 promotes proliferation and invasion of osteosarcoma cells by repressing PTEN expression. Many reports had shown the function of miR-490-3p in different cancers. Jia et al[19] found that miR-490-3p played a suppressive role in breast cancer cell proliferation, invasion by repressing the expression of TNKS2. Chen et al[20] showed that miR-490-3P might target CDK1 and inhibited ovarian epithelial carcinoma tumorigenesis and progression. Notably, Liu et al[13] reported that miR-490-3p modulates osteosarcoma cell proliferation *in vitro* and tumorigenicity *in vivo* by down-regulating HMGA2 expression directly. These results suggest that miR-490-3p may serve as an oncomiR in osteosarcoma.

Cancer-secreted microRNAs are investigated for their potential use as prognostic and predictive biomarkers. More studies proved that miRNAs could be a useful biomarker for cancer diagnosis and prognosis. For instance, Li et al[21] found that osteosarcoma patients with low miR-452 expression had a significantly shorter overall survival than those with high miR-452 expression. Wang et al[22] reported that down-regulation of miR-152 could be considered as a predictor for diagnosis and prognosis of osteosarcoma patients. A previous study has shown that miR-490-3p served as a tumor suppressor in osteosarcoma. However, the correlations between its expression and clinicopathological factors of osteosarcoma remain unclear.

In the present work, for the first time, we investigated the association of miR-490-3p expression with osteosarcoma progression and prognosis. Our results showed that miR-490-3p expression was significantly downregulated in osteosarcoma tissues compared with the adjacent noncancerous tissues. Kaplan-Meier survival curve analysis revealed that patients with high expression of miR-490-3p lived longer than those with low expression. In addition, univariate and multivariate analysis suggested that low miR-490-3p expression was an independent predictor of poor prognosis.

Conclusions

Our findings clearly indicated that miR-490-3p is involved in the progression of osteosarcoma and low miR-490-3p expression is associated with poor prognosis in osteosarcoma patients. These findings suggested that miR-490-3p might serve as a suitable prognostic marker for osteosarcoma patients.

Conflict of Interest

The Authors declare that there are no conflicts of interest.

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