

Original Article

Identification of microRNA-143 as a prognostic marker for survival in patients with osteosarcoma

Guo-Jun Wei^{1*}, Cao Li^{3*}, Meng Yao³, Yan-Song Wang¹, Peng-Zhen Lei¹, Hong-Wei Lei², Da-Ming Dong¹

¹Department of Orthopedics, The First Affiliated Hospital of Harbin Medical University, Harbin, Heilongjiang Province, China; ²Department of Rheumatology, The Second Affiliated Hospital of Harbin Medical University, Harbin, Heilongjiang Province, China; ³Department of Orthopedics, The Second Affiliated Hospital of Harbin Medical University, Harbin, Heilongjiang Province, China. *Equal contributors.

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Abstract: MicroRNA-143 (miR-143) expression has been demonstrated to be significantly decreased in osteosarcoma tissues. However, its clinical significance in osteosarcoma is still unclear. Therefore, the aim of this study was to investigate the association of miR-143 expression with clinicopathologic features and prognosis in patients with osteosarcoma. Quantitative real-time reverse transcriptase-polymerase chain reaction analysis was performed to detect expression levels of miR-143 in osteosarcoma and noncancerous bone tissues from 150 patients with primary osteosarcomas. Then, the clinical significance of miR-143 expression in osteosarcomas was determined. Compared with noncancerous bone tissues, the expression levels of miR-143 were significantly lower in osteosarcoma tissues ($P < 0.001$). Lower miR-143 expression occurred more frequently in osteosarcoma tissues with large tumor size ($P = 0.005$), advanced clinical stage ($P = 0.003$) and positive distant metastasis ($P = 0.008$). Moreover, the univariate analysis demonstrated that osteosarcoma patients with low miR-143 expression had poorer overall ($P < 0.001$) and disease-free survival ($P < 0.001$). Furthermore, the multivariate analysis identified low miR-143 expression as an independent prognostic factor for both overall ($P = 0.002$) and disease-free survival ($P = 0.004$) by Kaplan-Meier method and log-rank test. For the first time, the current data offer convincing evidence that the low miR-143 levels could serve as a prognostic indicator of poor survival and metastasis in osteosarcoma.

Keywords: Osteosarcoma, miR-143, survival, tumor metastasis

Introduction

Osteosarcoma is the most common histological form of primary bone cancer that affects mostly children and young adults [1]. Although many researchers have studied the etiology of osteosarcoma including epidemiologic and environmental factors and genetic impairments, it remains obscure [2, 3]. Treatment has evolved to include systemic chemotherapy and local control surgery [4]. Mifamurtide improves the overall survival from 70 to 78% and results in a one-third reduction in the risk of death from osteosarcoma [5, 6]. It is essential to screen novel molecular markers for the diagnosis, the prognosis, and the treatment of patients with osteosarcoma [7].

MicroRNAs (miRNAs), a class of small noncoding RNA molecules, can negatively modulate protein expression at the post-transcriptional

level [8, 9]. miRNAs regulate a variety of normal physiologic processes and are involved in tumorigenesis and development of multiple malignancies [10]. Recent genome-wide screening using miRNAs expression profiles has identified specific miRNAs expression patterns that are associated with the biological and clinical properties of cancers [11]. Several studies have demonstrated the involvement of miRNAs in the pathogenesis of osteosarcoma with the potential for development in disease diagnostics and therapeutics [11-16]. MicroRNA-143 (miR-143) expression has been demonstrated to be significantly decreased in osteosarcoma tissues [17-19]. However, its clinical significance in osteosarcoma is still unclear. Therefore, the aim of this study was to investigate the association of miR-143 expression with clinicopathologic features and prognosis in patients with osteosarcoma.

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Table 1. Association of miR-143 expression with clinicopathological features of osteosarcoma

Variable	Cases	miR-143 expression		P
		High (n, %)	Low (n, %)	
Age				0.96
<15	65	14	51	
≥15	85	18	67	
Gender				0.47
Male	88	17	71	
Female	62	15	47	
Tumor size				0.005
>8 cm	57	5	52	
≤8 cm	93	27	66	
Clinical stage				0.003
IIA	54	19	35	
IIB/III	96	13	83	
Tumor location				0.62
Extremities	122	27	95	
Other	28	5	23	
Histological type				0.87
Osteoblastic	82	17	65	
Chondroblastic	31	6	25	
Fibroblastic	20	5	15	
Telangiectatic	17	4	13	
Distant metastasis				0.008
Absent	109	30	79	
Present	41	2	39	
Response to chemotherapy				0.86
Good	63	13	50	
Poor	87	19	68	

Table 2. Multivariate survival analysis of overall survival and progression-free survival in 150 patients with osteosarcoma

Variables	Overall survival			Disease-free survival		
	RR	95% CI	P	RR	95% CI	P
miR-143 expression	5.3	1.5-11.3	0.002	4.7	1.4-9.5	0.004
Tumor size	2.3	1.2-6.1	0.02	2.1	1.1-5.7	0.04
Clinical stage	3.6	1.3-8.6	0.008	3.3	1.2-7.9	0.01
Distant metastasis	4.5	1.4-9.4	0.006	4.2	1.1-8.3	0.02

Materials and methods

Ethical statement

The Ethical Committee of the Second Affiliated Hospital of Harbin Medical University approved the study protocols, and all participants gave written informed consent according to the Declaration of Helsinki. All the methods applied in

the study were carried out in accordance with the approved guidelines.

Study population

This study was conducted in 150 patients with osteosarcoma during the years 2008 to 2015 in the Second Affiliated Hospital of Harbin Medical University, China. Osteosarcoma specimens and demographic, medical, and family histories were obtained from sequentially ascertained, unrelated patients. Patients with osteosarcoma were newly diagnosed and histopathologically confirmed independently by two gynecologic pathologists. Clinical and pathological information was extracted including tumor size, Enneking stage (I, II and III), tumor location (extremities and other), histological type (osteoblastic, chondroblastic, fibroblastic and mixed), tumor metastasis and response to chemotherapy.

RNA isolation

Total RNA isolation was performed using mirVana miRNA Isolation Kit (Applied Biosystems/Ambion, Austin, TX) according to the manufacturer's protocol. RNA concentrations were measured using the NanoDrop ND-1000 spectrophotometer (NanoDrop Technologies, Wilmington, DE, USA).

Quantitative real-time reverse transcriptase-polymerase chain reaction analysis

The expression of miR-143 was quantified using commercially available TaqMan qRT-PCR assays (Applied BioSystems, Foster City, CA) and a 7900HT Real-Time PCR System (Applied BioSystems, Foster City, CA). The reaction was carried out at 95°C for 10 min, 40 cycles of 95°C for 15 s, and 60°C for 1 min. All reactions were performed in triplicate, and expression levels of miR-143 was determined with the ΔCT method and reported as $2^{-\Delta\text{CT}}$. Tumor samples were classified into two different groups with high or low expression of miR-143 according to median level of normal bone tissues.

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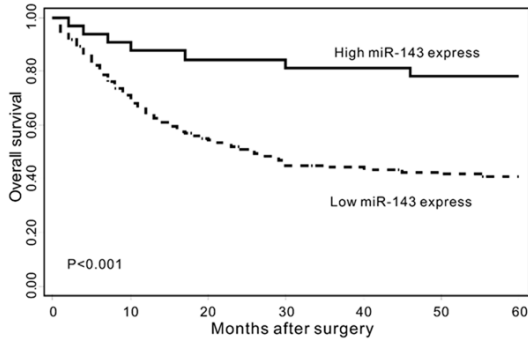


Figure 1. Overall survival curves for two groups defined by low and high expression of miR-214 in patients with pediatric osteosarcoma by Kaplan-Meier method and log-rank test.

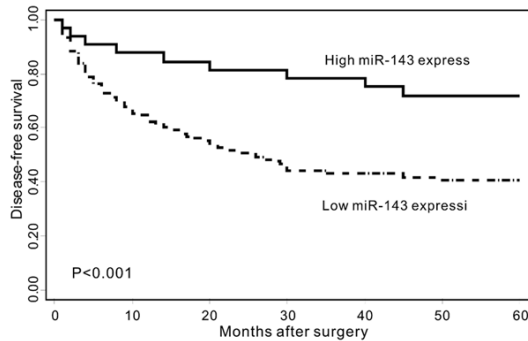


Figure 2. Disease-free survival curves for two groups defined by low and high expression of miR-214 in patients with pediatric osteosarcoma by Kaplan-Meier method and log-rank test.

Statistical analysis

Continuous variables were expressed as mean \pm standard deviation (SD). The Chi-squared test was used to show differences of categorical variables. Patient survival and their differences were determined by Kaplan-Meier method and log-rank test. Cox regression (proportional hazard model) was adopted for multivariate analysis of prognostic factors. $P<0.05$ was considered statistically significant. The statistical analysis was conducted using STATA 11.0 (StataCorp, College Station, Tex, USA).

Results

Compared with noncancerous bone tissues, the expression levels of miR-143 were significantly lower in osteosarcoma tissues ($P<0.001$). Lower miR-143 expression occurred more frequently in osteosarcoma tissues with large tumor size ($P=0.005$), advanced clinical stage

($P=0.003$) and positive distant metastasis ($P=0.008$) (**Table 1**). Moreover, the univariate analysis demonstrated that osteosarcoma patients with low miR-143 expression had poorer overall ($P<0.001$) and disease-free survival ($P<0.001$) (**Table 2**). Furthermore, the multivariate analysis identified low miR-143 expression as an independent prognostic factor for both overall ($P=0.002$) (**Figure 1**) and disease-free survival ($P=0.004$) (**Figure 2**) (**Table 2**) by Kaplan-Meier method and log-rank test.

Discussion

Several studies have investigated the effect of miR-143 on osteosarcoma. Hu *et al.* found that the miR-143 expression was confirmed to be downregulated in the MG-63 osteosarcoma cell line [18]. Zhang *et al.* found that miR-143, down-regulated in osteosarcoma, promoted apoptosis and suppressed tumorigenicity by targeting Bcl-2 [20]. Osaki *et al.* found that the downregulation of miR-143 correlated with the lung metastasis of human osteosarcoma cells by promoting cellular invasion, probably by regulating matrix metalloproteinase-13 expression [21]. Liu *et al.* found that circulating miR-143 could serve as a novel biomarker for osteosarcoma and was correlated with both metastasis status and histological subtype of the patients [17]. Exosome-formed synthetic miR-143 could be transferred to osteosarcoma cells and inhibited their migration [22]. A negative correlation between cyclooxygenase-2 and miR-143 might exist in the progression of osteosarcoma [23]. The miR-143 might play a crucial role in the chemoresistance of osteosarcoma tumors [24]. To investigate the association of miR-143 expression with clinicopathologic features and prognosis in patients with osteosarcoma, we conducted this study. We found that: (1) The expression levels of miR-143 were significantly lower in osteosarcoma tissues. (2) Lower miR-143 expression occurred more frequently in osteosarcoma tissues with large tumor size, advanced clinical stage and positive distant metastasis. (3) Osteosarcoma patients with low miR-143 expression had poorer overall and disease-free survival.

Our results mentioned above are similar to the findings in many other cancers. The miR-143 is a putative predictive factor for the response to fluoropyrimidine-based chemotherapy and a prognostic marker for survival in patients with

metastatic colorectal cancer [25]. Avgeris *et al.* uncovered the clinical utility of miR-143, miR-145 and miR-224 for predicting the survival of bladder cancer patients following treatment [26]. Naito *et al.* found that the miR-143 expression served as a prognostic marker of gastric cancer [27]. Down regulation of miR-143 is related with tumor size, lymph node metastasis and HPV16 infection in cervical squamous cell cancer [28].

However, the exact mechanism of miR-143 in osteosarcoma remains unknown. The miR-143 was down-regulated in osteosarcoma cell lines and primary tumor samples, and the restoration of miR-143 reduced cell viability, promoted cell apoptosis and suppressed tumorigenicity [20]. Additionally, Bcl-2, an important antiapoptotic molecule, was identified to be a novel direct target of miR-143, and the proapoptotic function of miR-143 was further suggested to be mainly through the targeting of Bcl-2 expression [20]. The downregulation of miR-143 correlated with the lung metastasis of human osteosarcoma cells by promoting cellular invasion, probably via matrix metalloproteinase-13 upregulation [21]. The miR-143 could regulate cancer glycolysis via targeting hexokinase 2 gene, coding for the first rate-limiting enzyme of glycolysis [29].

Two limitations should be cautioned. First of all, the sample size in our study was relatively smaller. Further experimental validation using a large number of tumor samples should be performed. Second, further research on the biological mechanism of the association of miR-143 expression with clinicopathologic features and prognosis in patients with osteosarcoma was necessary.

In conclusion, for the first time, our data offer convincing evidence that the low miR-143 levels could serve as a prognostic indicator of poor survival and metastasis in osteosarcoma.

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Disclosure of conflict of interest

None.

Address correspondence to: Da-Ming Dong, Department of Orthopedics, The First Affiliated Hospital of Harbin Medical University, 23 Youzheng Street, Harbin 150086, Heilongjiang Province, China. E-mail: dongdaming1965@hotmail.com

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