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Original article

Elevated expression of microRNA-19a predicts a poor prognosis in patients with osteosarcoma

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ABSTRACT

MicroRNA (miR)-19a, a member of the miR-17-92 cluster, functions as an oncomiRNA in multiple kinds of cancers. However, its involvement in human osteosarcomas remains unclear. In this study, to analyze the expression pattern of miR-19a and to investigate its clinical implication in human osteosarcomas, quantitative reverse-transcription polymerase chain reaction was performed to detect expression levels of miR-19a in 166 self-pairs of osteosarcoma and noncancerous bone tissues. Associations between miR-19a expression and various clinicopathological parameters and patients' prognosis of osteosarcomas were further evaluated. As a results, miR-19a expression in osteosarcoma tissues was significantly higher than that in corresponding noncancerous bone tissues ($P < 0.001$). Osteosarcoma patients with high miR-19a expression more frequently had large tumor size ($P = 0.03$), advanced clinical stage ($P = 0.01$), positive distant metastasis ($P = 0.008$) and poor response to chemotherapy ($P = 0.01$) than those with low miR-19a expression. Additionally, kaplan-Meier analysis showed that both overall and disease-free survivals of osteosarcoma patients with high miR-19a expression were shorter than those with low miR-19a expression (both $P < 0.001$). Further multivariate analysis identified miR-19a expression as an independent prognostic factor for both overall ($P = 0.001$) and disease-free ($P = 0.006$) survivals. In conclusion, the aberrant expression of miR-19a may play a crucial role in development and progression of human osteosarcomas. MiR-19a may act as a novel prognostic marker for patients with this malignancy.

1. Introduction

MicroRNAs (miRNAs), a novel class of short (18–25 nucleotides in length) and non-coding RNAs, function as regulators of gene expression by binding to complementary sites within the 3′-untranslated region (UTR) of target mRNAs [1]. MiRNAs are endogenously expressed in animal and plant cells, and have been indicated to play important roles in various cellular processes, including development, cell proliferation, cell cycle, apoptosis, cell differentiation and cell death [2]. Growing evidence show that miRNAs act either as oncogenes or tumor suppressors during the initiation and progression in a variety of human cancers [3]. Osteosarcoma represents the most common primary malignancy in bone tissues and is characterized by malignant osteoid production and malignant cells with osteoblastic differentiation [4]. Due to the early metastatic potential and poor prognosis, this aggressive bone tumor has become the leading cause of cancer-related death among children and adolescents [5]. In recent years, there have been great advances in tumor diagnosis and treatment including surgery, radiotherapy, and chemotherapy, however, the incidence of osteosarcoma is rising at a rate of 1.4% per year and the prognosis in patients with recurrence and metastasis remains poor [6]. Thus, it is an urgent need to identify reliable markers of early diagnosis and prognosis in order to improve the clinical outcome of human osteosarcoma. Accumulating studies have reported a large number of miRNAs which play crucial roles in the complex processes during progression of this malignancy. For example, increased expression of miR-1908 may be associated with poor clinical outcome in patients with osteosarcoma [7]; miR-409-3p may inhibit cell invasion and migration of osteosarcoma cells by targeting catenin-δ1 [8]; miR-198 can suppress tumorous behaviors of osteosarcoma through directly targeting ROCK1 [9]. These findings imply that the aberrant expression of miRNAs may be associated with tumor initiation and progression through the regulation of cancer-related genes and pathways involved in osteosarcoma pathogenesis.

MiR-19a, a member of the highly conserved miR-17-92 cluster located on chromosome 13q31.3, has been reported to act as an oncogenic miRNA in multiple cancer types [10]. MiR-19a expression has been observed to be increased in leukemia, esophageal squamous cell carcinoma, breast cancer, lung can-

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cer, colorectal cancer and cervical cancer, implying its association with tumor initiation of these malignancies [11–15]. Functionally, miR-19a suppresses apoptosis of laryngeal squamous cell carcinoma by regulating TIMP-2 expression [16]; miR-19a promotes cell growth of bladder cancer cells via targeting PTEN [17]; miR-19a plays inhibitory roles in malignancy of heaptocellular carcinoma via regulating Cyclin D1 expression [18]; miR-19a is associated with lymph metastasis and mediates the TNF-α induced epithelialto-mesenchymal transition in colorectal cancer [14]; miR-19a enhances cell growth and viability, cell invasion and migration in non-small cell lung cancer by targeting the suppressor of cytokine signaling 1 and mediating STAT3 activation [19]. However, the involvement of this miRNA in human osteosarcomas remains unclear. To address this problem, in the current study, we firstly detected expression levels of miR-19a in 166 self-pairs of osteosarcoma and noncancerous bone tissues by quantitative reverse-transcription polymerase chain reaction (qRT-PCR). Then, we evaluated the associations between miR-19a expression and various clinicopathological parameters and patients' prognosis of osteosarcomas statistically.

2. Materials and methods

2.1. Ethnic statement

This study was approved by the Research Ethics Committee of Shanghai 6th people's hospital, Shanghai Changhai hospital, Shanghai East hospital, Zhujiang Hospital, and Xuhui central hospital, Shangha, China. All patients enrolled in this study offered written informed consent. All tissue specimens obtained from patients with osteosarcomas were handled and made anonymous according to the ethical and legal standards.

2.2. Patients and tissue samples

This study used the same cohort of tissue specimens with our previous study [20]. A total of 166 patients with primary osteosarcomas were enrolled in this study from Shanghai 6th people's hospital, Shanghai Changhai hospital, Shanghai East hospital, Zhujiang Hospital, and Xuhui central hospital, Shangha, China, during 1998 and 2008. The osteosarcoma and the corresponding noncancerous bone tissues were collected from surgical specimens for qRT-PCR analysis. Our pathologist (F.S.) confirmed that the corresponding non-cancerous bone tissues had no tumor cells. No patients received blood transfusion, radiotherapy, or chemotherapy before surgery. Clinical stage of all 166 osteosarcoma patients were classified based on the 6th edition of the tumor-node-metastases (TNM) classification of the International Union against Cancer (UICC). The clinicopathological characteristics of these patients is provided in Table 1.

2.3. QRT-PCR

QRT-PCR assay was performed to detect the expression levels of miR-19a in osteosarcoma and the corresponding noncancerous tissues according to the protocol described in our previous study [20]. Primer sequences were presented as following: for miR-19a, RT: 5′-GTC GTA TCC AGT GCA GGG TCC GAG GTA TTC GCA CTG GAT ACG ACT CAG TTT-3′; Forward: 5′-CTG GAG TGT GCA AAT CTA TGC-3′; Reverse: 5′-GTG CAG GGT CCG AGG T-3′; U6, RT: 5′-AAA ATA TGG AAC GCT TCA CGA ATT TG-3′; Forward: 5′-CTC GCT TCG GCA GCA CAT ATA CT-3′; Reverse: 5′-ACG CTT CAC GAA TTT GCG TGT C-3′. The relative expression level of miR-19a was calculated using the $2^{-\Delta\Delta Ct}$ method, with the CT values normalized using U6 as an internal control.

Table 1
Associations between miR-19a expression and various clinicopathological characteristics of human osteosarcomas.

Clinicopathological features	No. of cases	miR-19a expression		P
		High (n, %)	Low (n, %)	
Age				
<55	116	60 (51.72)	56 (48.28)	NS
≥55	50	26 (52.00)	24 (48.00)	
Gender				
Male	96	48 (50.00)	48 (50.00)	NS
Female	70	38 (54.29)	32 (45.71)	
Tumor size (cm)				
>8	88	58 (65.91)	30 (34.09)	0.03
≤8	78	28 (35.90)	50 (64.10)	
Anatomic location				
Tibia/femur	103	56 (54.37)	47 (45.63)	NS
Elsewhere	63	30 (47.62)	33 (52.38)	
Serum level of lactate dehydrogenase				
Elevated	90	50 (55.56)	40 (44.44)	NS
Normal	76	36 (47.37)	40 (52.63)	
Serum level of alkaline phosphatase				
Elevated	108	60 (55.56)	48 (44.44)	NS
Normal	58	26 (44.83)	32 (55.17)	
Clinical stage				
IIA	38	8 (21.05)	30 (78.95)	0.01
IIB/III	128	78 (60.94)	50 (39.06)	
Distant metastasis				
Absent	96	28 (29.17)	68 (70.83)	0.008
Present	70	58 (82.56)	12 (17.44)	
Response to chemotherapy		86	80	
Good	68	16 (23.53)	52 (76.47)	0.01
Poor	98	70 (71.43)	28 (28.57)	

'NS' refers to differences without statistical significance.