Down-regulation of microRNA-182 and microRNA-183 predicts progression of osteosarcoma

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A b s t r a c t

Introduction: The aim of this study was to investigate the expression levels of microRNA-182 and microRNA-183 and their association with clinicopathological features in patients with osteosarcoma.
Material and methods: Total RNA was purified from samples and noncancerous bone tissues and then quantitative real-time polymerase chain reaction was applied to evaluate the expression levels of microRNAs, and their relationship with clinicopathological features and survival in osteosarcoma patients.
Results: Our findings showed that expression of MiR-182 was clearly lower in osteosarcoma bone tissue (mean ± SD: 2.84 ±.07) compared with noncancerous bone tissues (6.23 ±1.72, $p = 0.004$). On the other hand, lower expression of MiR-183 was seen in osteosarcoma bone tissue (1.43 ±0.59) when compared with normal tissues (4.36 ±2.47, $p = 0.036$). Decreased expression of MiR-182 was clearly correlated with advanced clinical stage ($p = 0.001$), metastasis or recurrence ($p = 0.024$), and large tumor size ($p = 0.032$). Decreased expression of MiR-183 was associated with advanced TNM stage ($p = 0.004$), and metastasis or recurrence ($p = 0.002$). A multivariate Cox proportional hazards model revealed that low expression of MiR-182 and MiR-183 ($p = 0.02$; $p = 0.016$), TNM stage ($p = 0.04$), and metastasis or recurrence ($p = 0.03$) were significantly associated with poor survival as independent prognostic factors.
Conclusions: These findings suggest that MiR-182 and MiR-183 may be associated with progression and metastasis of osteosarcoma.

Key words: tumor, oncology, pathology, marker, expression.

Introduction

Osteosarcoma commonly occurs in children and young adults as a primary bone malignancy [1–3]. It has been reported that 60% of malignant bone tumors occur in the first 2 decades of life. Nevertheless, a large

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number of osteosarcoma cases have responded poorly to chemotherapy. Furthermore, it has been suggested that the patients are at risk of distant metastasis or local relapse after curative surgery and chemotherapy [4, 5]. Despite the progress in therapeutic targets, they remain unsatisfactory for most osteosarcoma patients with metastasis or recurrent osteosarcoma. Therefore, discovery of new specific therapeutic targets may provide effective management of the disease. MicroRNAs belong to the class of small non-coding RNAs [6], and may act as a significant marker for prognosis and detection of cancer [7–9]. It has been suggested that microRNAs are correlated with cell fate specification, cellular proliferation, differentiation and apoptosis through alteration of the targets' expression [7, 10, 11].

Dysregulation of different microRNAs has been suggested in the context of osteosarcoma [12–15]. However, the role of MiRNAs in development of osteosarcoma remains ambiguous, and further studies are needed.

Therefore, our aim was to evaluate the expression pattern of MiR-182 and MiR-183 in human osteosarcoma and their association with clinicopathological factors.

Material and methods

Samples

Forty paired tissue samples of osteosarcoma and noncancerous bone tissue were collected from different hospitals in Tehran, Iran between March 2010 and February 2014 and were confirmed by the Research Ethics Committee. The diagnosis and the histological grading were approved by an independent pathologist. All the specimens were stored in liquid nitrogen after surgical operation until use. The clinicopathological features are presented in Table I.

Quantitative real-time PCR

The total RNA was isolated from frozen samples using TRIzol reagent based on the manufacturer's instructions. Gene-specific primers were used to synthesize cDNA from the TaqMan MicroRNA assays and reagents from the TaqMan MicroRNA Reverse Transcription kit (Applied Biosystems, Foster City, CA, USA). Real-time polymerase chain reaction was carried out to determine the expression level of microRNAs using an Invitrogen kit with the Rotor-gene 6000 system (Qiagen). The primers were used from the TaqMan MiRNA assays. The relative

Table I. Relationship of microRNA expression with clinicopathological factors of patients with osteosarcoma

Characteristic	N	MiR-182 expression		MiR-183 expression		P-value (MiR-182)	P-value (MiR-183)
		Low 21	High 19	Low 26	High 14		
Gender:							
Male	26	12	14	17	9	0.601	0.312
Female	14	9	5	9	5		
Age:							
≤ 40	29	15	14	19	10	0.523	0.414
> 40	11	6	5	7	4		
Tumor diameter [cm]:							
≤ 5	24	14	10	15	9	0.032	0.423
> 5	16	7	9	11	5		
Metastasis or recurrence:							
No	23	9	14	13	10	0.024	0.002
Yes	17	12	5	13	4		
Differentiation:							
High and moderate	21	10	11	12	9	0.123	0.113
Poor	19	11	8	14	5		
TNM stage:							
I + II	25	9	16	14	11	0.001	0.004
III + IV	15	12	3	12	3		

amount of microRNAs was normalized with the U6 gene as an internal reference. $\Delta\Delta$Ct ($\Delta\Delta$Ct = ΔCt $_{tumor\ samples}$ – ΔCt $_{control\ sample}$) was calculated to qualify the expression rate of MiR-182 and MiR-183.

Statistical analysis

We used SPSS 18.0 software for statistical analysis (SPSS Inc., USA). Differences between groups were evaluated using the χ^2 test. Survival analysis was performed using the log-rank test and Kaplan-Meier method. A Cox proportional hazards model was performed to evaluate prognostic values of clinicopathological factors. Differences were considered statistically significant when $p < 0.05$.

Results

Our findings showed that expression of MiR-182 was clearly lower in osteosarcoma bone tissue (mean ± SD: 2.84 ±0.07) compared with non-cancerous bone tissues (6.23 ± 1.72, $p = 0.004$; Figure 1), On the other hand, lower expression of MiR-183 was observed in osteosarcoma bone tissue (1.43 ±0.59) when compared with normal tissues (4.36 ±2.47, $p = 0.036$; Figure 2). According to the median expression level of the two mi-

croRNAs, we categorized the patients into low and high expression groups. The correlations between clinicopathological factors and expression of the two microRNAs in high and low expression groups are summarized in Table I.

The results showed that decreased expression of MiR-182 was clearly correlated with advanced clinical stage ($p = 0.001$), metastasis or recurrence ($p = 0.024$), and large tumor size ($p = 0.032$). No significant difference was found between MiR-182 and age ($p = 0.523$), gender ($p = 0.601$), or differentiation ($p = 0.123$) (Table I). Decreased expression of MiR-183 was associated with advanced TNM stage ($p = 0.004$), and metastasis or recurrence ($p = 0.002$). There was no significant correlation of MiR-183 with other clinical factors (Table I).

Kaplan-Meier survival and log-rank analysis were performed to evaluate the association of microRNA expression with survival of patients. As shown in Figures 3 and 4, the decreased expression of these microRNAs was strongly correlated with shorter overall survival (log-rank test $p = 0.035$; $p = 0.029$).

The multivariate Cox proportional hazards model revealed that low expression of MiR-182 and MiR-183 ($p = 0.02$; $p = 0.016$), TNM stage ($p = 0.04$), and metastasis or recurrence ($p = 0.03$)
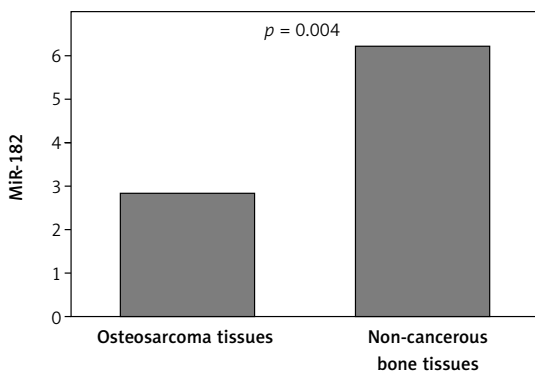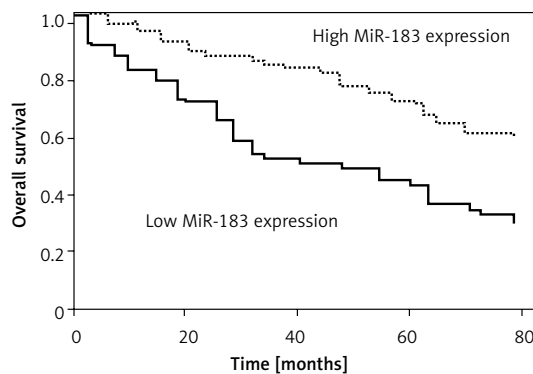

Figure 1. Relative expression level of MiR-182 between osteosarcoma bone tissue and noncancerous bone tissues
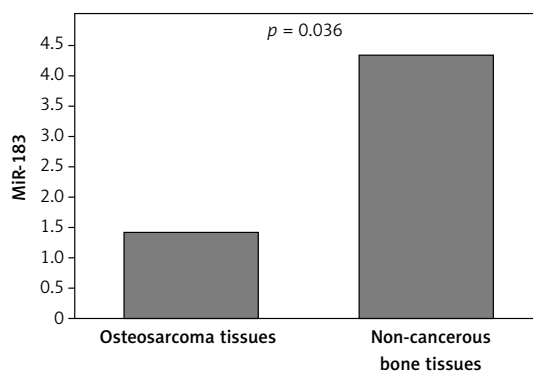
Figure 2. Relative expression level of MiR-183 between osteosarcoma bone tissue and noncancerous bone tissues
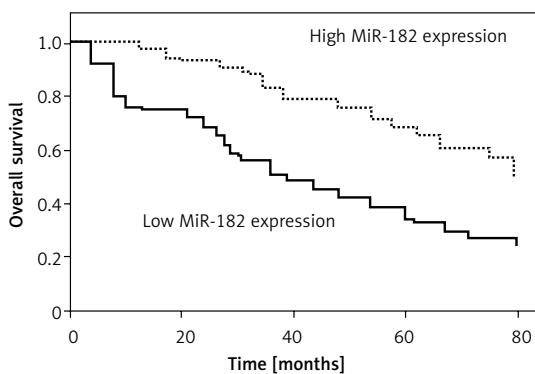
Figure 3. Correlation of MiR-182 expression with survival time in patients with osteosarcoma

Figure 4. Correlation of MiR-183 expression with survival time in patients with osteosarcoma

Table II. Multivariate analysis of the correlation of prognosis miR-182 with clinicopathological factors

Clinicopathological characteristics	HR	95% CI	*P*-value
Gender	0.535	0.752–1.827	0.63
Age	1.231	0.663–2.531	0.52
Anatomic location	1.027	0.231–1.863	0.68
TNM stage	2.754	2.024–7.612	0.04
Tumor size [cm]	3.129	1.934–9.723	0.03
Metastasis or recurrence	4.374	3.432–12.026	0.03
Differentiation	1.748	1.631–4.931	0.09
MiR-182 level	3.852	1.473–10.432	0.02

were significantly associated with poor survival as independent prognostic factors (Tables II and III).

Discussion

The role of MiRNAs in development of osteosarcoma remains ambiguous, and discovery of new specific therapeutic targets may provide effective management of the disease. Dysregulation of different MiRNAs has been previously suggested in many kinds of tumor [13, 14, 16, 17]. In the current study, we evaluated the expression pattern of MiR-182 and MiR-183 in relation to osteosarcoma.

Our findings showed that expression of MiR-182 and MiR-183 was clearly lower in osteosarcoma bone tissue compared with noncancerous bone tissues. Our findings suggest that their down-regulation is correlated with the progression of osteosarcoma and low expression of mentioned MiRNAs can contribute to tumor occurrence and development. Aberrant regulation of MiR-182 has been reported in many kinds of malignancies, including gastric cancer, lung, bladder, endometrial, prostate, colon and breast cancers, ovarian carcinoma, pediatric acute leukemia and melanoma [18, 19]. The tumor suppressor role of MiR-182 or oncogene has been suggested in different human malignancies. For instance, MiR-182 inhibits proliferation and invasion by targeting CTTN in lung adenocarcinoma cells [20], and could act as a tumor suppressor in lung tumor by targeting RGS17 [21]. In addition, the over-expression of MiR-182 has been previously reported, and also it was indicated that MiR-182 can play a role as an oncogene in different human cancers including melanoma [18].

Previous studies have suggested that MiR-183 can inhibit cell migration by targeting ITGB1 in neurosensory organs [22]. Moreover, MiR-183 can play a key role in initiation of tumor and progression in hepatocellular carcinoma cells [23]. Nevertheless, further investigations are needed to identify the role of MiR-183 in pathogenesis of osteosarcoma. Our results indicated that low expression of MiR-182 and MiR-183 was correlated with tumor progression in osteosarcoma. Decreased expression of MiR-182 was clearly correlated with advanced clinical stage, metastasis or recurrence, and high tumor size. Decreased expression of MiR-183 was associated with advanced TNM stage, and metastasis or recurrence.

It has been reported that MiR-182 was decreased in osteosarcoma tissues and cell lines.

Table III. Multivariate analysis of the correlation of prognosis miR-183 with clinicopathological factors

Clinicopathological characteristics	HR	95% CI	*P*-value
Gender	0.53	0.732–2.135	0.64
Age	1.102	0.543–2.983	0.54
Anatomic location	1.231	0.257–3.172	0.51
TNM stage	2.755	2.247–7.482	0.04
Tumor size [cm]	3.012	1.543–10.452	0.001
Metastasis or recurrence	4.618	3.155–13.026	0.01
Differentiation	1.892	1.821–4.367	0.08
MiR-183 level	4.183	1.643–11.521	0.02

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Up-regulation of MiR-182 can inhibit tumor growth, invasion and migration. Subsequent findings showed that TIAM1 was a direct target of MiR-182 in osteosarcoma cells [24]. On the other hand, it has been found that ectopic overexpression of MiR-183 repressed the expression levels of ezrin and markedly inhibited the motility and invasion of osteosarcoma cells. Recently, further studies suggested that MiR-183 may act as a tumor suppressor in the metastasis of osteosarcoma via downregulation of ezrin expression [25].

Kaplan-Meier survival and log-rank analysis indicated that decreased expression of these microRNAs was strongly correlated with shorter overall survival, which might be related to prognosis of osteosarcoma. The multivariate Cox proportional hazards model revealed that low expression of MiR-182 and MiR-183, TNM stage, and metastasis or recurrence were significantly associated with poor survival as independent prognostic factors.

In conclusion, our result showed that MiR-182 and MiR-183 may be associated with progression and metastasis of osteosarcoma. Further studies are needed to clarify the role of these markers.

Conflict of interest

The authors declare no conflict of interest.

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