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ORIGINAL ARTICLE

Downregulation of miR-185 and upregulation of miR-218 expression may be potential diagnostic and prognostic biomarkers of human chondrosarcoma

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Abstract Increasing evidence has confirmed that dysregulation of microRNAs (miRNAs) can contribute to the progression and metastasis of human tumors. Chondrosarcoma is the most common primary malignant bone tumor in adults and has no effective systemic treatment, and patients with this disease have poor survival. Thus, it is important to find new diagnostic markers and improve treatment options. In the current study, we are interested to examine the role of miR-185 and miR-218 expression in patients with chondrosarcoma using real-time PCR. Moreover, the association of the two miRNAs with clinicopathological features and prognosis was evaluated. Survival and Cox proportional hazards analyses were performed to find the association of miR-185 expression and miR-218 levels with prognosis in the patients. Our results indicated that the miR-185 expression was significantly downexpressed in clinical chondrosarcoma bone tissues compared with adjacent normal tissues (P=0.001). MiR-218 expression level was increased in clinical chondrosarcoma bone tissue than those adjacent normal tissues (P=0.001). Decreased expression of miR-185 showed remarkable correlation with advanced tumor stage (P=0.019), tumor grade (P<0.001), and distant metastasis (P=0.001). Moreover, high expression of miR-218 was strongly correlated with advanced tumor stage (P=0.014), tumor grade (P<0.001), and distant metastasis (P=0.002). Kaplan–Meier survival analysis revealed that the low miR-185 expression group and the high miR-218 expression group had remarkably shorter overall survival (log-rank test P=0.007, P=0.004). The multivariate Cox proportional hazards model indicated that decreased expression of miR-185 and increased expression of miR-218 (P=0.017, P=0.012), advanced tumor stage (P=0.006, P=0.012), tumor grade (P=0.032, P=0.016), and distant metastasis (P=0.004, P=0.015) were independently related to overall survival in patients with chondrosarcoma. In conclusion, downregulation of miR-185 and upregulation of miR-218 can be associated with progression of chondrosarcoma and also both of them may act as tumor suppressor genes in chondrosarcoma.

Keywords Chondrosarcoma · PCR · MicroRNAs · Prognostic · Pathology

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Introduction

Chondrosarcoma is known to be the second common primary bone malignant tumor after myeloma and osteosarcoma [1–3]. Patients with chondrosarcoma respond poorly to both radiation and chemotherapy and show

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poor prognosis after surgical resection because patients are at a distant chondrosarcoma metastasis risk and mortality results from distant metastasis. Therefore, identification of the importance of biomarkers and therapeutic targets for chondrosarcoma is required. MicroRNAs (miRNAs) belong to a class of non-coding RNAs, which can act as modulators of targeted gene expression. Moreover, miRNAs could contribute to tumorigenesis and cancer metastasis [4, 5], which makes miRNA an important target for cancer diagnosis and therapy. These data indicated that miRNAs might act as an oncogene or as a tumor suppressor. Dysregulation of different microRNAs has been demonstrated in chondrosarcoma [6–8]. However, the role of miRNAs in the development of chondrosarcoma remains ambiguous and further studies are needed.

In the current study, we evaluate the clinical importance of miR-185 and miR-218 expression in chondrosarcoma tissues using real-time PCR. Moreover, the associations of miR-185 and miR-218 levels with clinicopathological features were investigated.

Materials and methods

Patients and methods

In this study, primary central chondrosarcoma of bone specimens and adjacent normal tissues were obtained between 2009 and 2013 from 49 patients who were undergoing surgery in various hospitals in Tehran and Tabriz, Iran. Moreover, the diagnosis and the histological grading were confirmed by two pathologists. The detailed information of clinicopathological features is listed in Tables 1 and 2.

Quantitative real-time PCR

Total RNA was purified from tumoral and adjacent normal tissues using TRIzol reagent (Invitrogen, Carlsbad, CA). The TaqMan miRNA assay kit (Applied Biosystems) was used to evaluate the expression levels of miRNAs. Real-time PCR was performed using Rotor Gene 6000 Real-Time PCR (Qiagen, Germany) with an Invitrogen kit, and also a TaqMan

Table 1 The association of miR-185/miR-218 expression levels with characteristics of chondrosarcoma

Clinicopathological features	No. of cases	Expression of miR-185		Expression of miR-218		*P* value of miR-185	*P* value of miR-218
		Low=30	High=19	Low=23	High=26		
Gender							
Male	29	17	12	12	17	0.639	0.712
Female	20	13	7	11	9		
Age							
≤40	19	11	8	8	11	0.516	0.617
>40	30	19	11	15	15		
Primary site							
Pelvis	14	9	5	6	8	0.473	0.453
Scapula	9	5	4	6	3		
Femur	3	2	1	1	2		
Vertebra	9	6	3	4	5		
Rib	6	4	2	3	3		
Knee	4	3	1	1	3		
Tibia	4	1	3	2	2		
Tumor grade							
1	21	10	11	13	8	<0.001	<0.001
2	17	12	5	7	10		
3	11	8	3	3	8		
Distant metastasis							
Yes	15	13	2	4	11	0.001	0.002
No	34	17	17	19	15		
Tumor stage							
Ia	14	5	9	10	4	0.019	0.014
Ib	10	5	5	5	5		
IIa	6	6	0	2	4		
IIb	19	14	5	6	13		

Table 2 Multivariate analysis for prognostic factors (miR-185 expression)

Clinicopathological characteristics	HR	95 % CI	*P* value
Gender	0.824	0.378–1.931	0.731
Age	1.034	0.482–3.723	0.313
Tumor stage	3.416	1.817–10.563	0.006
Location	0.412	0.285–3.152	0.627
Distant metastasis	3.672	2.383–12.536	0.004
Tumor grade	2.294	1.32–8.431	0.032
MiR-185 expression	2.931	1.274–9.341	0.017

universal PCR master mix was used. RNA samples were normalized with U6 snRNA (an internal standard control). Fold change expression of miRNAs was calculated using the ΔΔ-CT method ($\Delta\Delta Ct=\Delta Ct_{\text{tumor samples}}-\Delta Ct_{\text{control sample}}$).

Statistical analysis

All statistical analysis was done using the SPSS 18.0 software (SPSS Inc., USA). All differences were evaluated by Student's *t* test. The correlation between miRNA expression and the different clinicopathological characteristics was analyzed using the χ^2 test.

Survival times were counted to the date of death, and survival rate analysis was detected using the log-rank test and Kaplan–Meier method. In addition, a Cox proportional hazards model was performed to evaluate prognostic values of the clinicopathological factors. $P<0.05$ was statistically significant.

Results

MiR-185 was downregulated in chondrosarcoma tissues

Our result indicated that the miR-185 expression was significantly downexpressed in clinical chondrosarcoma bone tissues compared with adjacent normal tissues (2.257±1.423 vs. 7.312±1.835, $P=0.001$).

MiR-218 was overexpressed in chondrosarcoma tissues

The miR-218 expression level was increased in clinical chondrosarcoma bone tissues than that in adjacent normal tissues (12.526±3.305 vs. 2.524±1.241, $P=0.001$).

Correlation of miRNA expression with the clinicopathological features

The patients with chondrosarcoma were categorized into low- and high-expression groups based on the median value of relative miRNA expression. Decreased expression of miR-185 showed a remarkable correlation with advanced tumor stage ($P=0.019$), tumor grade ($P<0.001$), and distant metastasis ($P=0.001$). No significant association was found between miR-185 and age ($P=0.516$), gender ($P=0.639$), and location ($P=0.473$; Table 1).

Moreover, the high expression of miR-218 was strongly correlated with advanced tumor stage ($P=0.014$), tumor grade ($P<0.001$), and distant metastasis ($P=0.002$), but no significant relationship with other clinical factors (Table 2).

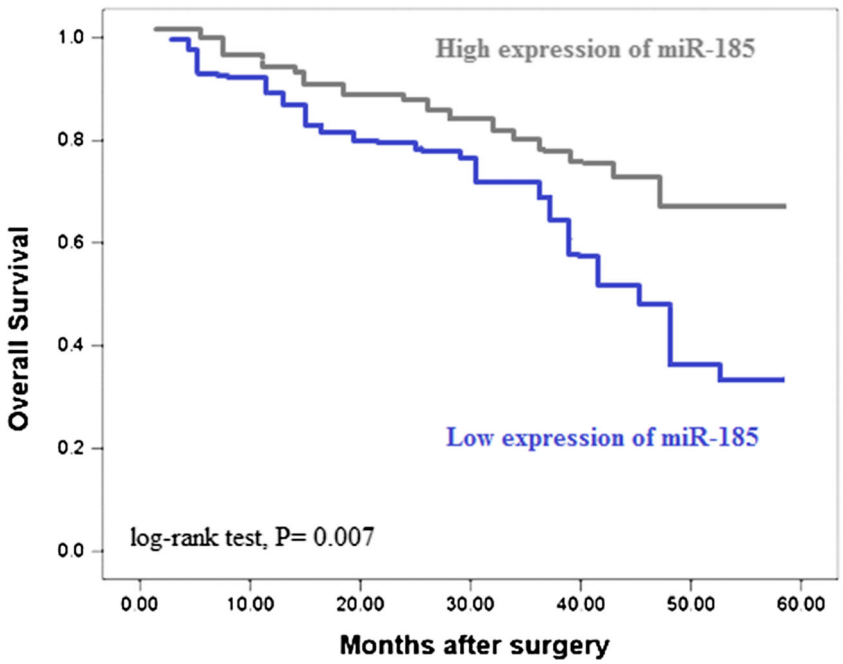

Fig. 1 Correlation between miR-185 expression level and overall survival time

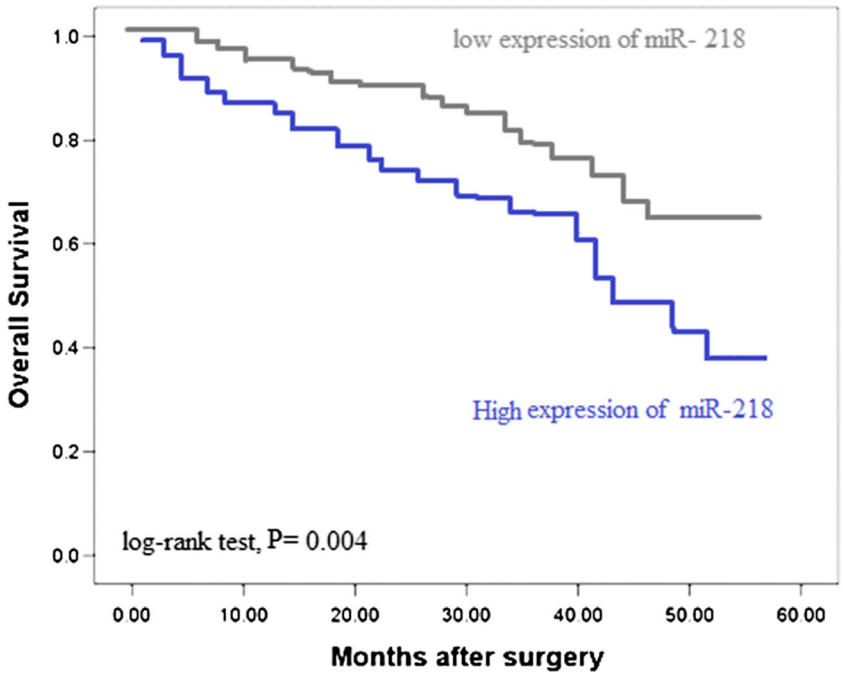

Fig. 2 Correlation between miR-218 expression and overall survival time

Kaplan–Meier survival analysis revealed that the low miR-185 expression group and the high miR-218 expression group had shorter overall survival (log-rank test P=0.007, P=0.004; Figs. 1 and 2). The multivariate Cox proportional hazards model indicated that decreased expression of miR-185 and increased expression of miR-218 (P=0.017, P=0.012), high tumor stage (P=0.006, P=0.012), tumor grade (P=0.032, P=0.016), and distant metastasis (P=0.004, P=0.015) were independently related to overall survival of patients suffering from chondrosarcoma (Tables 2 and 3).

Discussion

MiRNAs are involved in tumorigenesis and cancer metastasis and might act as an oncogene or as a tumor suppressor [4, 5]. The result indicated that downregulation of miR-185 and upregulation miR-218 can be associated with progression of osteosarcoma.

Table 3 Multivariate analysis for prognostic factors (miR-218 expression)

Clinicopathological characteristics	HR	95 % CI	*P* value
Gender	0.973	0.894–2.012	0.631
Age	1.293	0.627–4.832	0.3
Tumor stage	3.241	1.623–10.862	0.012
Location	0.647	0.381–3.012	0.629
Distant metastasis	3.062	1.93–12.283	0.015
Tumor grade	3.233	1.174–9.073	0.016
MiR-218 expression	3.234	1.274–9.163	0.012

Dysregulation of different microRNAs has been demonstrated in chondrosarcoma [6–9]. However, the role of miRNAs in the development of chondrosarcoma remains ambiguous and further studies are needed. Nevertheless, the biological function of miR-185 in patients with chondrosarcoma and its association with clinical factors have not been fully explained. Therefore, in the present study, we evaluate the clinical importance of miR-185 and miR-218 expression in chondrosarcoma tissues using real-time PCR. Our result indicated that miR-185 was significantly downexpressed in clinical chondrosarcoma bone tissues compared with adjacent normal tissues. Decreased expression of miR-185 showed a remarkable correlation with high tumor stage, tumor grade, and distant metastasis. Our result suggested that decreased expression of miR-185 may be linked to progression of chondrosarcoma. Decreased expression of miR-185 has been reported in ovarian cancer, prostate cancer cases, breast cancer tissues, and pediatric renal tumors, and it was suggested that this decreased expression of miR-185 may contribute to tumor initiation and progression [10–12]. It has been suggested that overexpression of miR-185 can inhibit the cell proliferation in breast cancer cells by regulating the c-Met expression, suggesting a therapeutic potential of miR-185 for treatment of breast cancer. It can be interpreted that miR-185 might act as an oncogene or a suppressor of tumor in many kinds of cancers, depending on cellular context.

On the other hand, the miR-218 expression level was increased in clinical chondrosarcoma bone tissues than that in adjacent normal tissues. Overexpression of miR-218 was

strongly correlated with high tumor stage, tumor grade, and distant metastasis. These results suggested that overexpression of miR-218 may be linked to progression of chondrosarcoma.

Dysregulation of miR-218 has been indicated in different kinds of cancer [13, 14]. It has been suggested that miR-218 acts as a tumor suppressor gene in many kinds of cancer by targeting many genes to regulate biological processes [15–17].

Overexpression of miR-218 can inhibit cancer cell proliferation, invasion, and metastasis, and also promote cancer cell apoptosis [16, 17]. Leite et al. (2011) suggested that the miR-218 expression level was higher in high-grade prostate cancer when compared with metastatic prostate cancer [18]. MiR-218 may play an important role in the progression of colorectal cancer (CRC) [19].

Kaplan–Meier survival analysis revealed that the low miR-185 expression group and the high miR-218 expression group had a remarkably shorter overall survival. The multivariate Cox proportional hazards model indicated that decreased expression of miR-185 and increased expression of miR-218, high tumor stage, tumor grade, and distant metastasis were independently related to overall survival of patients suffering from chondrosarcoma.

Budhu et al. (2008) suggested that decreased expression of miR-185 correlates with more tumor recurrence and a short survival time in comparison with the higher miR-185 expression group, and they concluded that miR-185 can inhibit cell growth and invasion in vitro in patients with hepatocellular carcinoma, indicating that miR-185 may act as a tumor suppressor gene in HCC patients [19]. It was found that low miR-218 expression was associated with a short survival time in patients with colorectal cancer and lung cancer [19, 20]. According to previous studies, it can be interpreted that aberrant expression of miR-218 in specific tissues can be an independent prognostic factor for overall survival of patients. Our result suggested that these microRNAs may be prognostic markers in patients suffering from chondrosarcoma.

In conclusion, downregulation of miR-185 and upregulation of miR-218 can be associated with progression of chondrosarcoma and also both of them may act as tumor suppressor genes in chondrosarcoma.

Conflicts of interest None

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