Short Communication

Diagnostic and prognostic values of blood microRNA-Let7A for osteosarcoma

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ABSTRACT

Objective: In view of the poor prognosis and difficulties in the diagnosis of osteosarcoma, and the functionality of microRNA-Let7A in different types of human cancers, our study aimed to explore the diagnostic and prognostic values of microRNA-Let7A for osteosarcoma.

Methods: A total of 39 patients with osteosarcoma and 19 normal healthy people were included in this study. All patients received surgical resection, and tumor tissues as well as pericarcinomatous tissues were collected during surgical operation. Venous blood (2 ml) was extracted from each participant. Expression of microRNA-Let7A in tumor tissues and pericarcinomatous tissues, and expression of E2F2 and microRNA-Let7A in blood of each participant was detected by qRT-PCR. ROC analysis was performed to evaluate the diagnostic values of blood E2F2 and microRNA-Let7A for osteosarcoma, and prognostic values of microRNA-Let7A for osteosarcoma was evaluated by survival curve comparisons.

Results: Expression level of microRNA-Let7A was significantly lower in tumor tissues than that in pericarcinomatous tissues. MicroRNA-Let7A expression in blood was significantly downregulated in osteosarcoma patients compared with normal control. Expression of microRNA-Let7A was negatively correlated with the expression of E2F2 in blood of osteosarcoma patients. Compared with E2F2, blood microRNA-Let7A can more effectively predict osteosarcoma. Overall survival rate of osteosarcoma patient with low blood expression level of miRNA-let-7a was significantly lower than that of patients with high blood expression level of miRNA-let-7a.

Conclusion: Blood microRNA-Let7A is a promising diagnostic and prognostic biomarker for osteosarcoma.

1. Introduction

As a type of malignancy that develops from bone tissues, osteosarcoma mainly affects teenagers and young adults [1]. Osteosarcoma is a relatively rare type of cancer. In the United States, only 1000 new cases of this disease were reported every year [2]. In spite of the low incidence, osteosarcoma now is considered to be one of the major causes of cancer-related death among children and young adults [3]. A varity of internal and external factors have been proved to be involved in the development of osteosarcoma. Those factors include familial inheritance such as deletion of chromosome 13q14, bone diseases such as enchondromatosis and fibrous dysplasia, and exposure to low doses of Sr-90 emission [4]. In spite of those progresses that have been made on the mechanism of osteosarcoma, pathogenesis of this disease still have not been fully elucidated, leading to the unsatisfactory treatment outcomes and poor prognosis. Up to now, early diagnosis and treatment is still the key for the treatment of this disease.

As a group of small non-coding RNAs with a length of 22–24 nucleotides, miRNAs have been proved to participate in nearly all aspects of critical biological processes including cell proliferation, differentiation and fibrosis [5–7]. Studies in last several years have shown that different miRNAs play different roles in the onset, development and progression of osteosarcoma [8,9]. MicroRNA-Let7A has been proved to play a role as tumor suppressor gene in several types of cancers such as Ewing's sarcoma [10] and thyroid cancer [11] and showed promising diagnostic and prognostic values for those diseases. However, the functionality of microRNA-Let7A in osteosarcoma, and the diagnostic as well as prognostic values of microRNA-Let7A for this disease still haven't been well studied.

In this study, expression of microRNA-Let7A in tumor tissues and pericarcinomatous tissues, as well as expression of E2F2 and microRNA-Let7A in blood of both osteosarcoma patients and healthy controls was

detected. Diagnostic and prognostic values of blood microRNA-Let7A for osteosarcoma were also evaluated.

2. Materials and methods

2.1. Patients

A total of 39 patients with osteosarcoma that enrolled in the Seventh People's Hospital of Suzhou from January 2010 to January 2012 were selected to serve as osteosarcoma group (OS group). Those patients included 20 males and 19 females, and the age ranged from 13 years to 68 years, with and average age of 34 ± 7.2 years. Inclusion criteria: patients did not receive radiotherapy and chemotherapy, patients with normal cardiopulmonary, liver and kidney function. Exclusion criteria: Patients with chronic diseases such as high blood pressure, chronic heart disease, and kidney failure. Among those patients, 10 showed no tumor metastasis, 12 showed lymph node metastasis without distant metastasis and 17 showed distant metastasis. At the same time, 19 healthy people were selected to serve as control group. Control group included 8 males and 11 females, and age ranged from 19 to 71 years, with an average age of 37 ± 9.1 years. There were no significant differences in age and gender between patients and control group.

2.2. Specimen collection

All patients received surgical resection, and tumor tissues as well as pericarcinomatous tissues within the area 2 cm around the tumors were collected during surgical operation. Venous blood (2 ml) was extracted through elbow vein from each participant and it was stored in anticoagulant tubes. All specimens were stored at $-80°C$ before use.

2.3. QRT-PCR

Total RNA was extracted from blood, tumor tissues and pericarcinomatous tissues using Trizol reagent (Invitrogen, USA) according to the instructions. RNA concentration was determined by NanoDrop™ 2000 Spectrophotometers (Thermo Fisher Scientific, USA). RNA samples with a A260/A280 ratio between 1.8 and 2.0 were subjected to reverse transcription to synthesize cDNA Oligo (dT) 15 (Sangon, Shanghai) and AMV reverse transcriptase (GIBCO, USA). MiRNA-let-7a was detected by TaqMan probe (5′-GAGGTAGTAGGTTGTATA-3′, TaqMan Micro-RNA Assay kit, Applied Biosystems), and miRNA-191 was used as the endogenous control. The following primers were used in PCR reactions: 5′- GAGCTCACTCAGACCCCAAG −3′ (forward) and 5′-AACAGGCTGAAGCCAAAAGA-3′ (reverse) for E2F2; 5′- GAGTCAAC GGATTTGGTCGT-3′ (forward) and 5′- TTGATTTTGGAGGGATCTCG-3′ (reverse) for GAPDH. PCR reaction conditions were: 95°C for 1 min, followed by 40 cycles of 95°C for 22 s and 60°C for 36 s. Ct values were processed using $2^{-\Delta\Delta CT}$ method. Relative expression level of each gene was normalized to endogenous control GAPDH.

2.4. Statistical analysis

SPSS19.0 (SPSS Inc., USA) was used to perform the analysis. Normal distribution data were recorded by ($\overline{x} \pm s$), and comparisons between two groups were performed using t test. Non-normal distribution data were analyzed using non-parametric Mann-Whitney U test. Correlation analysis was performed by Pearson correlation analysis. $P < 0.05$ was considered to be statistically significant.

3. Results

3.1. MiRNA-let-7a expression in OS group and control group

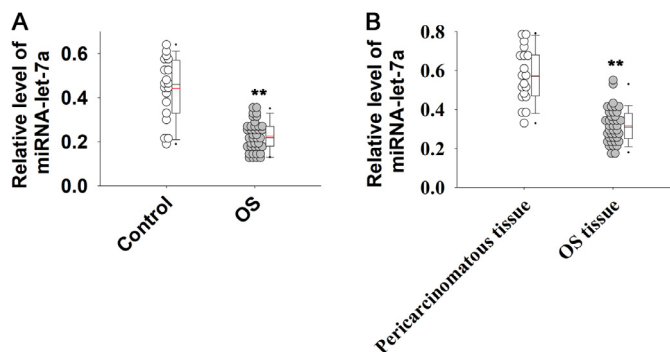We first measured the expression level of miRNA-let-7a in blood of

Fig. 1. Expression of miRNA-let-7a in OS and control groups. (A) Expression of miRNA-let-7a in blood of OS group and control group; (B) Expression of miRNA-let-7a in OS tissues and pericarcinomatous tissues.
Notes: **compared with control group or pericarcinomatous tissue, $p < 0.01$.

both OS group and control group. Results showed that expression level of miRNA-let-7a was significantly lower in OS group than that in control group ($p < 0.01$) (Fig. 1A). In addition, expression level of miRNA-let-7a was significantly lower in tumor tissues than that in pericarcinomatous tissues ($p < 0.01$) (Fig. 1B). Those data suggest that downregulation of miRNA-let-7a is very likely to be involved in the development of osteosarcoma.

3.2. E2F2 expression in blood of OS group and control group

MiRNA-let-7a can inhibit the activity of E2F2 to inhibit the growth of tumor cells in various types of cancer. Therefore, E2F2 expression in the blood of OS group and control group was detected. As shown in Fig. 2, expression level of E2F2 was significantly higher in OS group than that in control group ($p = 0.046$) (Fig. 2). Those data suggest that E2F2 expression is also likely to be involved in the development of osteosarcoma.

3.3. Correlation between miRNA-let-7a expression and E2F2 expression in blood and osteosarcoma tissues

It has been well established that miRNA-let-7a can downregulate the expression of E2F2. Therefore, correlation between miRNA-let-7a expression and E2F2 expression in blood and osteosarcoma tissues was analyzed. As shown in Fig. 3, miRNA-let-7a expression was negatively correlated with E2F2 expression in blood (Fig. 3A) and osteosarcoma tissues (Fig. 3B) of osteosarcoma patients.

3.4. Diagnostic values of blood miRNA-let-7a level for osteosarcoma

ROC curve analysis was performed to evaluate the diagnostic values of blood miRNA-let-7a level for osteosarcoma. As shown in Fig. 4, the area under the curve of miRNA-let-7a was 0.90, which was significantly bigger than that of E2F2 (0.65, $p = 0.019$), indicating that miRNA-let-
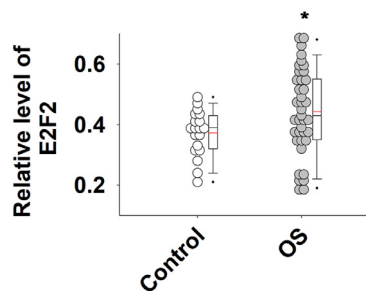

Fig. 2. E2F2 expression in blood of OS group and control group.
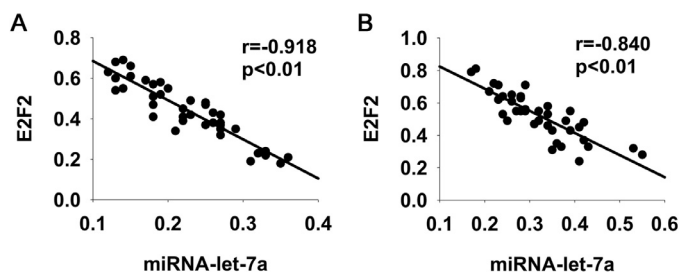*Notes:* *compared with control group, $p = 0.046$.

Fig. 3. Correlation between miRNA-let-7a expression and E2F2 expression in blood and osteosarcoma tissues.
This figure shows the correlations between miRNA-let-7a expression and E2F2 expression in blood (A) and osteosarcoma tissues (B).
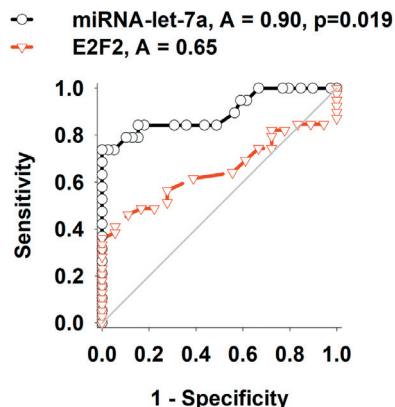

Fig. 4. ROC curve analysis to evaluate the diagnostic values of miRNA-let-7a and E2F2 for osteosarcoma.

7a can potentially serve as a promising biomarker for osteosarcoma.

3.5. Prognostic values of blood miRNA-let-7a level for osteosarcoma

According to the median blood miRNA-let-7a level, patients were divided into high expression group ($n = 20$) and low expression group ($n = 19$). Survival curves were plotted using Kaplan–Meier method. Survival curves were compared by log-rank test. As shown in Fig. 5, overall survival rate of osteosarcoma patient with low blood expression level of miRNA-let-7a was significantly lower than that of patients with high blood expression level of miRNA-let-7a ($p < 0.05$). Those results suggest that blood miRNA-let-7a may serve as a promising prognostic marker for osteosarcoma.
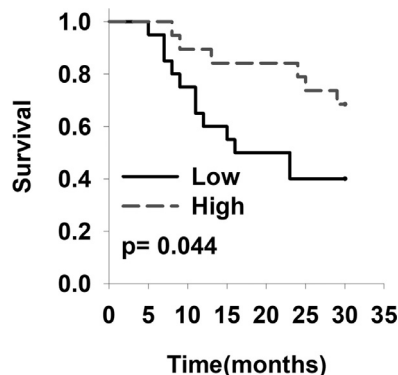

Fig. 5. Survival curves of osteosarcoma patients with high and low blood miRNA-let-7a level.

4. Discussion

The development of osteosarcoma is a complex process with various internal and external factors that involved. Advances in studies on pathogenesis of osteosarcoma have identified a variety of risk factors for osteosarcoma [12]. However, progresses in understanding of mechanism for the onset, development and progression of osteosarcoma also raised more questions. Studies even showed that the occurrence of osteosarcoma was also seriously affected by individual's height [13], indicating the complexity of the pathogenesis for this disease. The unclear pathogenesis is always one of the major challenges for the treatment of osteosarcoma [14]. MiRNAs play pivotal roles in almost all critical processes as well as pathological processes in human body [15]. Recent studies have found that the development of osteosarcoma also required the involvement of different miRNAs. In a recent study, Jin et al showed that miRNA-218 was downregulated in osteosarcoma, and miRNA-218 overexpression inhibited the migration and invasion of osteosarcoma cells through the downregulation of TIAM1, MMP2 and MMP9 expression [16]. In another study, miRNA-646 expression was found to be downregulated in osteosarcoma, while upregulation of miRNA-646 expression inhibited tumor metastasis through the inhibition of fibroblast growth factor 2 expression, indicating the role of miRNA-646 as a tumor suppressor in osteosarcoma [17]. In contrast, compared with adjacent healthy tissues, expression of microRNA-214 was upregulated in osteosarcoma tissues, and the increased expression level of microRNA-214 was closely correlated with the poor prognosis of those patients, indicating the role of microRNA-214 as an oncogene in osteosarcoma [18]. As a tumor suppressor gene, microRNA-Let7A expression is usually downregulated in various types of human cancer, such as Ewing's sarcoma [10] and thyroid cancer [11]. However, to date, expression pattern of microRNA-Let7A in osteosarcoma still has not been reported. In this study, expression level of microRNA-Let7A was found to be significantly lower in tumor tissues than that in pericarcinomatous tissues. In addition, blood level of microRNA-Let7A was also significantly lower in blood of osteosarcoma patients than that in normal control. Those data suggest that microRNA-Let7A is very likely to play a role as tumor suppressor in osteosarcoma.

Numerous studies have shown that microRNA-Let7A can achieve its biological functions in various human diseases including different types of cancers by targeting E2F2 to downregulate its expression. In the study of prostate cancer, results of both in vivo and in vitro experiments showed that microRNA-Let7A could inhibit the proliferation of prostate cancer cells by downregulating E2F2 expression [19]. In another study, microRNA-Let7A was proved to reduce the proliferation rate of osteosarcoma cells by targeting E2F2 [20]. Consistent with previous studies, in this study, blood level of microRNA-Let7A was found to negatively correlate with blood level of E2F2 in patients with osteosarcoma, which further confirmed the functionality of microRNA-Let7A in osteosarcoma.

The development of disease conditions is usually accompanied with the changes in certain substances of blood including miRNA, and detecting changes of those substances may provide valuable information for the diagnosis and prognosis of certain diseases [21]. Significant changes in genome-wide miRNA expression profile have been found in human osteosarcoma [22], and a considerable number of miRNAs have been proved to be of diagnostic and prognostic values for osteosarcoma [23]. In this study, ROC curve analysis was performed to evaluate the diagnostic values of microRNA-Let7A for osteosarcoma, and the results showed that the area under the ROC curve was 0.90, indicating the high accuracy and reliability of blood microRNA-Let7A as a diagnostic marker for osteosarcoma. In addition, survival curves that plotted by Kaplan-Meier method also showed that overall survival rate of osteosarcoma patient with low blood expression level of miRNA-let-7a was significantly lower than that of patients with high blood expression level of miRNA-let-7a. Those results suggest that blood miRNA-let-7a may serve as a promising diagnostic and prognostic marker for

osteosarcoma.

5. Conclusion

In conclusion, expression level of microRNA-Let7A was significantly downregulated in tumor tissues compared with pericarcinomatous tissues. MicroRNA-Let7A expression was also significantly downregulated in blood of osteosarcoma patients compared with healthy controls. Blood microRNA-Let7A can accurately and effectively predict osteosarcoma. Overall survival rate of osteosarcoma patient with low blood expression level of miRNA-let-7a was significantly lower than that of patients with high blood expression level of miRNA-let-7a. Our data indicate that blood miRNA-let-7a has the potentials to serve as a diagnostic and prognostic marker for osteosarcoma. However, our study is still limited by the small sample size. Further studies with bigger sample size are needed to further confirm the conclusion.

Acknowledgment

Conflict of interests

None declared.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.jbo.2018.05.001.

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