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Low-expression of lncRNA FER1L4 might be a prognostic marker in osteosarcoma

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Abstract. – OBJECTIVE: To investigate the potential role of Long non-coding RNA (lncRNA) FER1L4 in the diagnosis and prognostic assessment of osteosarcoma.

PATIENTS AND METHODS: LncRNA FER1L4 expression in osteosarcoma samples was detected by real-time PCR. The Kaplan-Meier method was used to analyze the relationship between lncRNA FER1L4 expression and the survival time of patients.

RESULTS: LncRNA FER1L4 expression was decreased in osteosarcoma samples. LncRNA FER1L4 was not related to the gender and age of patients, but was significantly associated with disease stage, metastasis, and tumor differentiation.

CONCLUSIONS: Low-expression of lncRNA FER1L4 might be a prognostic marker in osteosarcoma.

Key Words:
LncRNA FER1L4, Prognosis, Over survival, Osteosarcoma.

Introduction

Osteosarcoma is the most common bone tumor in adolescents. It mostly occurs in the metaphysis of long bones which grow fast, and 50%-70% occur around the knee joint. The incidence is about 6-8/1 million[1,2]. The prognosis is poor with a 5-year survival rate of about 70%. Several patients have been found in advanced or pulmonary metastasis[3]. Although advances have been made in the etiology, development, diagnosis, and treatment of osteosarcoma, the lack of effective indicators of diagnosis and treatment of osteosarcoma, such as serum diagnostic markers, has a profound impact on osteosarcoma[4].

Long non-coding RNA (lncRNA) is a non-coding RNA transcript that is more than 200 nucleotides in length and lacks an open reading frame encoding a protein, and differs from any known class of non-coding RNA. Studies[5,6] showed that lncRNA has some similarities with some genes encoding proteins, such as those transcriptional products produced by RNA polymerase II transcription complexes. LncRNA is widely transcribed in the genome and plays an important role in gene regulation, but its potential mechanism remains unclear. The expression of lncRNA is associated with a range of human diseases, such as cancer and neurological diseases. LncRNA is also involved in cell proliferation, differentiation and other activities, and the occurrence of cancer[7,8]. However, the functional role of lncRNA in osteosarcoma pathogenesis, disease diagnosis and treatment still remains uncertain. In this work, lncRNA FER1L4 expression was detected by Real-time quantitative PCR in osteosarcoma tissues and its corresponding paracancerous specimens, and its relationship with clinicopathological features was analyzed, so as to investigate the potential role of lncRNA FER1L4 in the diagnosis and prognostic assessment of osteosarcoma.

Patients and Methods

Specimens

All the samples were stored in liquid nitrogen for 30 minutes after surgery. The study was approved by the Ethics Committee of Yidu Central Hospital of Weifang hospital. All the patients signed the informed consent. All the patients with osteosarcoma obtained in our study were followed-up.

RNA Extraction

Total RNA was extracted from osteosarcoma and adjacent noncancerous tissues using TRIzol (Invitrogen, Grand Island, NY, USA) according to the manufacturer's protocol. SmartSpec Plus spectrophotometer (Bio-Rad, Hercules, CA,

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USA) was used to evaluate the RNA quantity. Based on the A260/A280 ratio all the RNA purity was examined.

Real-Time Quantitative PCR Analysis

The extracted total RNA was reverse transcribed into cDNA according to the instructions of the reverse transcription kit, and PCR amplification was performed on a Real-time PCR instrument according to the instruction manual of SYBR Green PCR Master (TaKaRa, Dalian, China), GAPDH acted as an internal reference. The PCR conditions were as follows: 95°C for 10 min (95°C for 15 s, 60°C for 30 s, 72°C for 30 s) × 40 cycles. The FER1L4 primers were as follows: forward, 5′-CCGTGTTGAGGTGCTGTTC-3′; reverse, 5′-GGCAAGTCCACTGTCAGATG-3′. The GAPDH primers were as follows: forward, 5′-AAGGTGAAGGTCGGAGTCAA-3′; reverse, 5′-AATGAAGGGGTCATTGATGG-3′. The experiments were conducted for three times. The relative quantification of gene expression was performed.

Statistical Analysis

The results were analyzed using the statistical product and service solutions (SPSS) 17.0 statistical software (SPSS Inc., Chicago, IL, USA). All quantitative data were expressed as mean ± standard deviation. Comparison between groups was done using One-way ANOVA test followed by post hoc test (Least Significant Difference). The relationship between lncRNA FER1L4 expression and clinical features was analyzed by Chi-Square test. $p<0.05$ or $p<0.01$ indicated that the difference was statistically significant.

Results

LncRNAFER1L4 Was Downregulated in Osteosarcoma Tissues

To explore the expression of lncRNA FER1L4 in osteosarcoma tissues and the adjacent non-cancerous samples, a Real-time PCR method was conducted. Notably, the results showed that the expression level of lncRNA FER1L4 in osteosarcoma tissues was significantly lower than that in adjacent non-cancerous tissues ($p<0.01$, Figure 1). This data indicated that the low-expression of lncRNA FER1L4 might contribute to the osteosarcoma progression.

LncRNA FER1L4 Expression Was Related to the Clinicopathological Features

With the combination of the clinical information, the relationship between lncRNA FER1L4 expression and the patient's clinicopathological features, including age, gender, disease stage, metastasis, and tumor differentiation, was analyzed. The results showed that the expression of lncRNA FER1L4 was not related to the gender and age, but was significantly associated with disease stage, metastasis, and tumor differentiation (Table I), suggesting that the dysregulation of lncRNA FER1L4 was implicated in the malignant characteristics.

Table I. Expression levels of lncRNA FER1L4 in osteosarcoma.

Characteristics	lncRNA FER1L4 All Patients	lncRNA low expression	FER1L4 high expression	p
No.	73	37	36	
Age (yr)				0.417
≤60	33	15	18	
>60	40	22	18	
Gender				0.724
Male	36	19	17	
Female	37	18	19	
Tumor differentiation				0.011
Poor/median	44	17	27	
High	29	20	9	
Disease stage				0.001
T1-2	43	15	28	
T3-4	30	22	8	
Metastasis				0.002
No	48	18	30	
Yes	25	19	6	

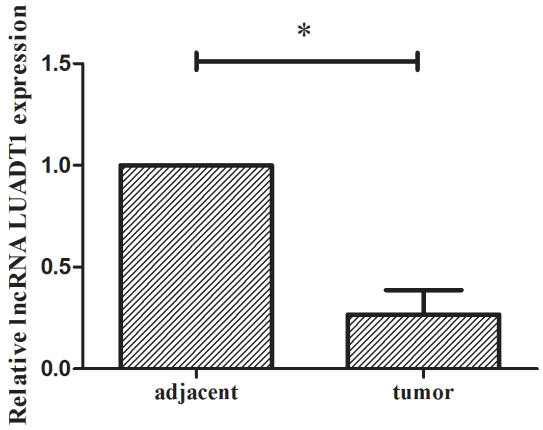

Figure 1. The RT-PCR analysis showed that lncRNA LUADT1 expression in tumor tissues was lower than that in the adjacent tissues. *p<0.05.

Low-expression of LncRNA FER1L4 Might Be a Prognostic Marker in Osteosarcoma

The Kaplan-Meier method was used to analyze the relationship between lncRNA FER1L4 expression and the prognosis of patients. The results showed that PFS (progression-free survival) in patients with high expression of lncRNA FER1L4 was higher than that in patients with low expression of lncRNA FER1L4 (p<0.01, Figure 2). Moreover, the OS (overall survival) of patients with low expression of lncRNA FER1L4 was lower than that of those with higher expression, the difference was statistically significant (p<0.01, Figure 3). In summary, all the results demonstrated that the low-expression of lncRNA FER1L4 might be a prognostic marker in osteosarcoma.

Discussion

Osteosarcoma is a malignant tumor originated from mesenchymal tissue, characterized by cells that can produce bone-like tissue. It is a malignant tumor with extremely high mortality and morbidity. The specific pathogenesis of osteosarcoma is not yet known clear[9,10].

As a newly non-coding RNA in recent years, lncRNA has become a hotspot in the field of tumor biology. It has been reported[11] that lncRNA UCC promotes the progression of colorectal cancer by inhibiting the expression of miR-143. HOTAIR promotes breast cancer metastasis by inducing chromatin rearrangements[12]. LncRNA is also found to be involved in the regulation of osteosarcoma development[13]. LncRNA EWSAT1

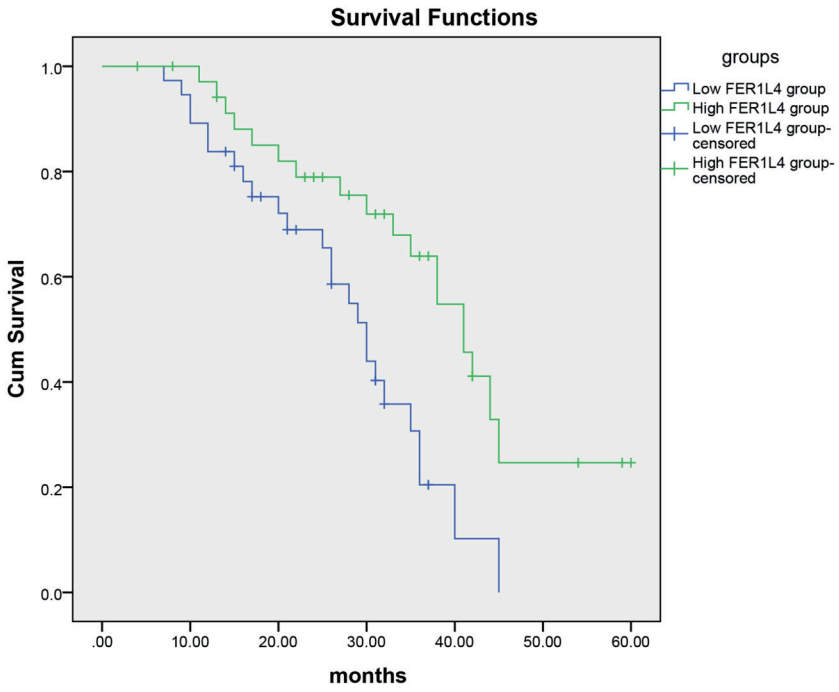

Figure 2. The Kaplan-Meier method analyzed that PFS in patients with high expression of lncRNA FER1L4 was higher in patients with low expression.

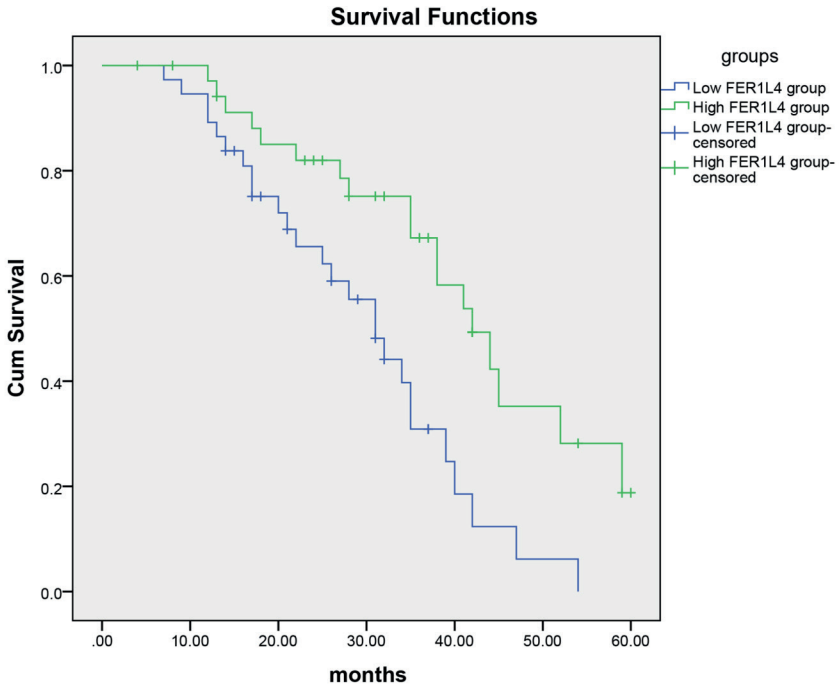

Figure 3. The Kaplan-Meier method analyzed that OS of patients with low expression of lncRNA FER1L4 was lower than that of those with higher expression.

promotes osteosarcoma cell growth and metastasis by inhibiting the expression of MEG3[14]. Up-regulation of lncRNA MFI2 can promote the proliferation of osteosarcoma cells and inhibit its apoptosis[15]. LncRNA BCAR4 promotes osteosarcoma progression by activating GLI2-dependent gene transcription[16].

LncRNA FER1L4 has been identified to function as a cancer-inhibitor in different cancers. For example, lncRNA FER1L4 could inhibit oncogenesis and acts as a prognostic marker via interfering with miR-106a in colon cancer[17]. Another study[18] showed that lncRNA FER1L4 could inhibit cell growth through regulating PTEN expression. In endometrial carcinoma, lncRNA FER1L4 could suppress cell proliferative ability and cell cycle via targeting PTEN (27381864)[19]. In glioblastoma, lncRNA FER1L4 was up-regulated and could control the glioma cells tumorigenicity[20]. LncRNA FER1L4 could predict prognosis in HCC[21]. However, the role of lncRNA FER1L4 in osteosarcoma has not been well studied yet. In this study, through the detection of lncRNA FER1L4 in 73 cases of osteosarcoma tissues and adjacent tissues, the relationship between lncRNA FER1L4 expression and clinicopathological characteristics of the patients were analyzed. The results showed that the expression of lncRNA FER1L4 in patients with osteosarcoma was reduced. The expression of lncRNA FER1L4 was significantly correlated with the stage of disease, metastasis, and tumor differentiation, but not with gender and age. The low expression of lncRNA FER1L4 was related to PFS (progression-free survival) and OS (overall survival) of the patients and might be a prognostic marker in osteosarcoma.

Conclusions

We showed that lncRNA FER1L4 was involved in the regulation of osteosarcoma development and may be used as a potential molecular marker for the diagnosis and prognosis of osteosarcoma.

Conflict of Interest

The Authors declare that they have no conflict of interest.

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