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Long non-coding RNA MALAT1 is an independent prognostic factor of osteosarcoma

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Abstract. – OBJECTIVE: Accumulating evidence revealed that long non-coding RNAs (lncRNAs) were emerging regulators in cancer biology, and could be used as potential biomarkers for cancer prognosis. In this study, we focused on MALAT1 and investigated its expression pattern, clinical significance in osteosarcoma.

PATIENTS AND METHODS: The expression of lncRNA MALAT1 was analyzed in 162 osteosarcoma tissues by quantitative real-time PCR (qRT-PCR). Then, we explored the potential relationship between MALAT1 expression levels in tumor tissues and clinicopathological features of osteosarcoma, and clinical outcome.

RESULTS: We found that which was significantly up-regulated in osteosarcoma tissues compared with paired non-tumor tissues ($p < 0.01$). The expression of MALAT1 was remarkably associated with advanced clinical stage and distant metastasis of osteosarcoma patients ($p < 0.05$). The Kaplan-Meier survival analysis showed that osteosarcoma patients with higher levels of MALAT1 had a shorter survival time. The multivariate Cox regression analysis demonstrated that MALAT1 expression level was an independent prognostic factor for the overall survival rate of osteosarcoma patients.

CONCLUSIONS: Our results demonstrated the clinical prognostic significance and roles of MALAT1 in osteosarcoma, and suggested that MALAT1 may be considered as a prognostic biomarker and therapeutic target for osteosarcoma.

Key Words
Long noncoding RNA, MALAT1, Osteosarcoma, Prognosis.

Introduction

Osteosarcoma is the most common primary sarcoma of bone in children and young adults, which accounting for 2.4% of all malignancies in pediatric patients and 20% of all primary bone cancers[1,2]. It is an aggressive bone tumor characterized by malignant osteoid production and malignant cells with osteoblastic differentiation[3].

Although improvements in therapeutic strategies including radiotherapy, adjuvant chemotherapy, and wide tumor excision were achieved, the outcome remains poor for most patients with metastatic or recurrent osteosarcoma[4,5]. Thus, the identification of novel prognostic biomarkers and potential therapeutic targets is crucial for improving the prognosis of osteosarcoma patients.

Long noncoding RNA (lncRNA), endogenous RNA gene products consisting of 200 to 100,000 nucleotides, was identified as important regulators of malignancies[6,7]. A large number of studies have shown that the disorders of lncRNA are closely related to human diseases, including various kinds of cancer[8,9]. Various lncRNAs play a crucial role in carcinogenesis: for example, Zhang et al[10] found that the long noncoding RNA AFAP1-AS1 is significantly up-regulated in hepatocellular carcinoma tiss ues, and regulate hepatocellular carcinoma cell invasion and metastasis, partially via the up-regulation of the RhoA/Rac2 signaling. Xie et al[11] showed that decreased lncRNA SPRY4-IT1 contributed to gastric cancer cell metastasis partly via affecting epithelial-mesenchymal transition. Li et al[12] found that the overexpression of long noncoding RNA HOTAIR leads to a chemoresistance by activating the Wnt/β-catenin pathway in human ovarian cancer. However, to our knowledge, the clinical significance and biological function of lncRNA MALAT1 in osteosarcoma remains unclear.

In the present study, we explored MALAT1 expression pattern and its correlation with clinicopathological features in osteosarcoma. Then, its prognostic significance was assessed. Our study highlighted the significance of MALAT1 in predicting patients' clinical outcome.

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Patients and Methods

Patients and Tissue Samples

A total of 162 primary osteosarcoma and corresponding noncancerous bone tissue samples were collected from the Linyi People's Hospital for RT-qPCR analysis between May 2008 and February 2014. All specimens were handled and made anonymous according to the ethical and legal standards. None of the patients received preoperative chemotherapy or radiotherapy before surgery. Clinical stage of these osteosarcoma patients was classified according to the sixth edition of the tumor-node-metastases (TNM) classification of the International Union Against Cancer (UICC). The surgically removed tissues were collected and immediately placed in liquid nitrogen and then stored at −80 °C until analysis. The clinicopathological features are summarized in Table I. The present study was approved by the Research Ethics Committee of Linyi People's Hospital, and written informed consent was obtained from all the patients.

Quantitative Real-time PCR Assay

Total RNA was isolated from tissue using TRIZOL reagent according to the manufacturer's protocol (Invitrogen Co, Carlsbad, CA, USA). RNA was reverse transcribed into cDNA using the Prime-Script one step RT-PCR kit (Takara, Dalian, Liaoning, China). The expression level of MALAT1 was detected by qPCR using the Ultra SYBR Mixture with ROX (Invitrogen Co, Carlsbad, CA, USA) and ABI7500 system (Applied Biosystems Life Technologies, Foster City, CA, USA). Results were normalized to the expression of GAPDH. The primers (Invitrogen) were designed as follows: for human MALAT1, the forward primer was 5′-AAAGCAAGGTCTCCCCACAAG-3′ and the reverse primer was

5′-GGTCTG TGCTAGATCAAAAGGCA-3′. For human GAPDH, the forward primer was 5′-CCCACTCCTCCACCTTTGAC-3′ and the reverse primer was

5′-ATGAGGTCCACCACCCTGTT-3.

All experiments were performed using the $2^{-\Delta\Delta Ct}$ method. Each experiment was performed in triplicate.

Statistical Analysis

All statistical analyses were performed using SPSS for Windows v.16.0 (SPSS, Chicago, IL, USA). Data are presented as mean ± standard deviation (SD). The statistical significance was

Table I. Correlation between MALAT1 expression and clinicopathologic features in patients with osteosarcoma.

Variables	Cases (n=162)	MALAT1 expression level		*p*-value
		Low expression	High expression	
Age (years)				0.202
<20	114	54	60	
≥20	48	28	20	
Gender				0.335
male	73	40	33	
female	89	42	47	
Tumor size (cm)				0.344
<8 cm	116	56	60	
≥8 cm	46	26	20	
Anatomic location				0.193
tibia/femur	113	61	52	
elsewhere	49	21	28	
Clinical stage				0.000
IIA	70	49	21	
IIB/III	92	33	59	
Distant metastasis				0.001
absence	118	69	49	
presence	44	13	31	

tested by the Student's *t*-test or the chi-square test as appropriate. Survival curves were plotted using the Kaplan-Meier method and the log-rank test. Independent prognostic indicators were assessed in the multivariate analysis using Cox's proportional hazard model. The results were considered to be statistically significant at $p < 0.05$.

Results

LncRNA MALAT1 is highly expressed in osteosarcoma

To explore the role of lncRNA MALAT1 in osteosarcoma, we performed qRT-PCR to examine MALAT1 expression levels in 164 clinical fresh samples of osteosarcoma tissues and non-malignant tissues. The results showed that MALAT-1 expression was significantly higher osteosarcoma tissues than in non-malignant tissues (Figure 1).

The Relationship between MALAT1 Expression and Clinicopathological Features in Osteosarcoma Patients

The 164 osteosarcoma patients were classified into two groups according to the median expression level of MALAT1: 82 patients were in the high expression of MALAT1 group, and 80 patients were in the low expression of MALAT1 group. As shown in Table I, the high MALAT1 expression level was observed to be closely correlated with advanced clinical stage and distant metastasis ($p < 0.05$). However, the high MALAT1 expression was not associated with other clinicopathological factors of osteosarcoma patients, including gender, age, tumor size and anatomic location.

Association between MALAT1 Expression and Survival in Osteosarcoma Patients

To explore the prognostic value of the lncRNA MALAT1 expression for osteosarcoma, the Kaplan-Meier analysis and log-rank test were performed to investigate the association between the levels of lncRNA MALAT1 expression and overall survival. The results revealed that the prognosis of osteosarcoma patients with high MALAT1 expression was significantly poorer than those with low MALAT1 expression (Figure 2; $p < 0.001$). In addition, we performed the univariate and the multivariate analysis to determine whether MALAT1 expression and other clinical parameters are independent factors for prognostic prediction in osteosarcoma patients. The results of analysis are shown in Tables II. The univariate analysis showed that MALAT1 expression ($p = 0.001$), clinical stage ($p = 0.005$) and distant metastasis ($p = 0003$) were significantly correlated with overall survival of OS patients. Moreover, the multivariate analysis confirmed that MALAT1 expression was an independent prognostic indicator for overall survival in osteosarcoma patients.

Discussion

Osteosarcoma, as the most common primary sarcoma of bone, is the leading cause of cancer-related death among children and adolescents[13]. The reliable identification of osteosarcoma progres-

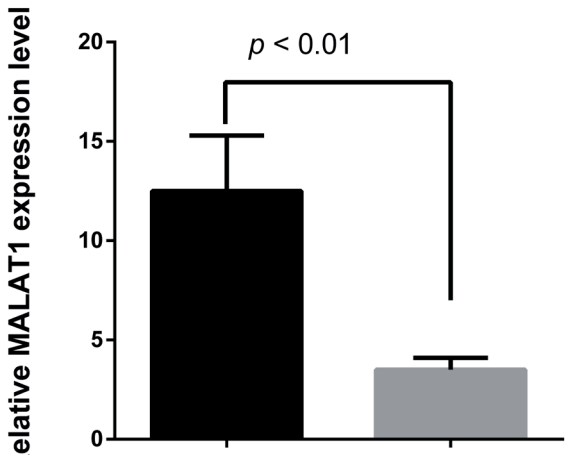

Figure 1. The relative expression of MALAT1 in osteosarcoma tissues, compared with noncancerous bone tissues (p < 0.01). Data were presented as the mean ± SEM of three independent experiments.

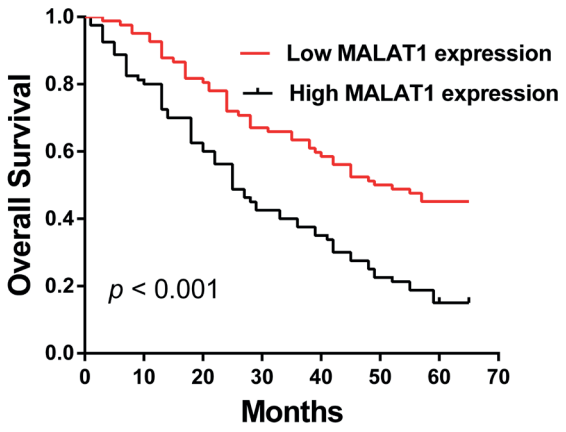

Figure 2. Correlation between MALAT1 expression and survival in patients.

Table II. Univariate and multivariate analysis of overall survival in osteosarcoma patients.

Variables	Univariate analysis			Multivariate analysis		
	Hazard ratio	95% CI	*p*	Hazard ratio	95% CI	*p*
Age (years)						
≥25 vs. <25	0.821	0.562-2.774	0.455			
Gender						
male vs. female	1.417	0.894-3.227	0.397			
Tumor size						
≥8 cm vs. <8 cm	2.261	0.665-5.948	0.078			
Anatomic location						
elsewhere vs. tibia/femur	0.744	0.618-2.554	0.341			
Clinical stage						
IIB/III vs. IIA	3.219	2.216-7.335	0.005	2.654	2.129-6.228	0.008
Distant metastasis						
presence vs. absence	4.391	2.165-8.885	0.003	4.152	1.656-7.778	0.006
MALAT1						
high vs. low	2.799	1.763-7.841	0.001	3.157	1.556-6.883	0.003

sion-specific targets has huge implications for its prevention and treatment[14]. However, developing new diagnostic and prognostic tools and effective therapeutics that may be beneficial for improving the clinical management of osteosarcoma remains a challenge.

LncRNA MALAT1, also known as nuclear-enriched abundant transcript 2 (NEAT2), is a highly abundant and ubiquitously expressed long ncRNA with a length of ~8000 nt[15]. Several studies reported that MALAT1 play an important role in several types of human cancer. For example, Zhang et al[16] found that MALAT1 was up-regulated in cervical cancer tissues compare to non-tumor tissues. Additionally, they found that MALAT1 promoted cell proliferation, invasion and migration. Ren et al[17] showed that MALAT1 significantly regulated cell growth and mobility, and also served as a poor prognostic factor for prostate cancer. In osteosarcoma, Cai et al[18] found that MALAT1 expression was up-regulated in human osteosarcoma cell lines and tissues, and knockdown of MALAT1 by siRNA significantly inhibited the cell proliferation and migration. Those results informed that MALAT1 might play important roles in osteosarcoma progression.

In the present study, MALAT1 expression levels were significantly higher in the osteosarcoma tissue. Moreover, we further identified the role of lncRNA MALAT1 in the development and progression of MALAT1. High MALAT1 expression was proved to be associated with clinical stage and distant metastasis. Furthermore, the overall survival time of patients with higher MALAT1 expression levels was shorter than that of patients with lower MALAT1 expression levels. In multivariate analysis, we confirmed that tumor MALAT1 was an independent significant prognostic factor. To the best of our knowledge, this is the first study to investigate the relationship between MALAT1 expression level and the prognosis of osteosarcoma patients.

Conclusions

Our work showed that the expression of MALAT1 was increased in osteosarcoma and associated with advanced tumor progression and unfavorable prognosis. These results suggested that MALAT1 could be employed as a new prognostic marker for breast cancer.

Conflicts of interest

The authors declare that no conflicts of interest exist.

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