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High levels of long non-coding RNA DICER1-AS1 are associated with poor clinical prognosis in patients with osteosarcoma

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Abstract. – OBJECTIVE: Long noncoding RNA DICER1-AS1 (DICER1-AS1) has been reported to be upregulated in osteosarcoma cells and to serve as a tumor promoter. However, the clinical significance of DICER1-AS1 in osteosarcoma remains unclear. The aim of this study was to investigate the association of DICER1-AS1 expression with prognosis of osteosarcoma.

PATIENTS AND METHODS: The expression of DICER1-AS1 was measured in 214 osteosarcoma samples and normal bone samples by using Real-time PCR. The correlations between DICER1-AS1 expression and clinical features were statistically analyzed. Overall survival (OS) and disease-free survival (DFS) were examined using Kaplan-Meier curves. Multivariate analyses were performed to analyze the prognostic significance of DICER1-AS1 expression.

RESULTS: It was found that the expression levels of DICER1-AS1 in osteosarcoma tissues were significantly higher than those in corresponding noncancerous bone tissues ($p < 0.01$). Higher DICER1-AS1 had significant association with clinical stage ($p = 0.005$) and distant metastasis ($p = 0.000$). Kaplan-Meier survival analysis showed that patients with high DICER1-AS1 expression had a shorter OS and DFS compared with the low DICER1-AS1 expression group ($p = 0.007$ and $p < 0.0001$). In a multivariate Cox model, our results showed that DICER1-AS1 expression was an independent poor prognostic factor for both 5-year OS (HR = 3.236, 95% CI: 1.148-5.347; $p = 0.004$) and 5-year DFS (HR = 3.935, 95% CI: 1.556-6.349; $p = 0.001$).

CONCLUSIONS: DICER1-AS1 is up-regulated in osteosarcoma and may serve as a potential prognostic biomarker for osteosarcoma.

Key Words:
LncRNA DICER1-AS1, Osteosarcoma, Prognosis.

Introduction

Osteosarcoma is a debilitating, high-grade primary bone malignancy affecting rapidly growing bones, and is responsible for 20% of all primary bone sarcomas[1,2]. Osteosarcoma is the most frequent primary bone malignant tumor and is often identified in children between the age of 10 and 20 years[3]. With the advancement of multiple therapeutic strategies for osteosarcoma including surgical resection, adjuvant chemotherapy and radiotherapy, the 5-year survival rate of osteosarcoma patients has been dramatically improved[4,5]. However, the prognosis of osteosarcoma patients with metastases is rather poor, and the long-term survival rate is only 10-30%[6]. Therefore, examining the pathogenesis and biological features of osteosarcoma is crucial to enhance early detection and treatment. In the human genome, approximately 2% of transcripts can be translated into proteins, whereas 98% of transcripts are noncoding RNAs (ncRNAs)[7]. Long noncoding RNA (lncRNA), >200 nucleotides in length, is a member of the non-coding RNA family that can regulate gene expression in transcriptional or posttranscriptional level[8]. Growing studies[9-11] show that lncRNAs could control various cellular processes, including proliferation, differentiation, apoptosis and metastasis, and are implicated in human diseases. More importantly, emerging evidence indicates that lncRNAs may play complex and extensive roles in promoting the tumorigenesis and progression of tumors[12,13]. In addition, aberrantly expressed lncRNAs can be detected in tumor tissues, and their expression profiles are different in different types of tumor. These findings indicate lncRNAs as suitable biomarkers to be used for osteosarcoma diagnosis and prognosis[14,15]. Recently, lncRNA DICER1-AS1 (DICER1-AS1), a novel tumor-related lncRNA, was reported to be abnormally expressed in osteosarcoma cells by lncRNA microarray and RT-PCR. Further cells experiments showed that

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DICER1-AS1 may function as a tumor promoter in osteosarcoma progression[16]. Up to date, this is the only study on the expression and biological function of DICER1-AS1 in tumor. However, whether DICER1-AS1 expression was dysregulated in osteosarcoma tissues and its clinical significance in osteosarcoma patients, remain largely unknown. Our present aimed to detect DICER1-AS1 expression in osteosarcoma patients and its potential as a prognostic biomarker for osteosarcoma patients.

Patients and Methods

Patients

A total of 214 clinical samples were obtained from patients with osteosarcoma and paired adjacent nontumor bone tissue at The Affiliated Huaian No.1 People's Hospital of Nanjing Medical University. All specimens were snap frozen in liquid nitrogen immediately after surgery and stored at -80°C. None of the patients had previously received radiotherapy, chemotherapy, or immunotherapy. The stage of the disease was classified according to the pathological Tumor-Node-Metastasis staging system. The follow-up information of all the participants was updated every 3 months for 5 years. This study was approved by the Research Ethics Committee of The Affiliated Huaian No.1 People's Hospital of Nanjing Medical University. Written informed consent was obtained from all of the patients.

RNA Extraction and RT-PCR

Total RNA was extracted with the TRIzol reagent (Invitrogen, Carlsbad, CA, USA) following the manufacturer's instructions. cDNA was reverse transcribed using the QuantiTect Reverse Transcription Kit (Qiagen, Hilden, Germany). Quantitative reverse transcription polymerase chain reaction (qRT-PCR) was conducted using a SYBR Premix Ex Taq II Kit (Thermo Fisher Scientific, Waltham, MA, USA) on an Applied Biosystems 7500 Real-Time PCR system (Thermo Fisher Scientific, Waltham, MA, USA). The relative levels of DICER1-AS1 were normalized to the expression of U6 by the $2^{-\Delta\Delta Ct}$ method. The primers used in this study were synthesized by GenePharma Company (Xuhui, Shanghai, China) and the sequences were shown in Table I.

Statistical Analysis

All data were analyzed SPSS 17.0 using the software package (SPSS Inc., Chicago, IL, USA). Data were analyzed using independent two-tailed *t*-test. The paired-samples t-test was used in the analysis of differential DICER1-AS1 expression between tumor and normal tissues. Associations between clinicopathological parameters and DICER1-AS1 expression were evaluated using chi-square tests. The overall survival (OS) and disease-free survival (DFS) were estimated using the Kaplan-Meier method. The significance of survival variables was evaluated using a multivariate Cox proportional hazards regression analysis. A p-value < 0.05 is considered statistically significant.

Results

Increased Expression of DICER1-AS1 in Human Osteosarcoma Tissues

To reveal the role of DICER1-AS1 in progression of osteosarcoma, we performed the qRT-PCR to evaluate the expression of DICER1-AS1 in 214 osteosarcoma and corresponding non-tumor tissues. We found that relative expression of DICER1-AS1 normalized to U6 in renal cancer was found to be 3.89 ± 0.47, while that in matching adjacent normal specimens was 7.67 ± 0.93. Statistical results indicated that DICER1-AS1 expression was upregulated in osteosarcoma tissues compared with normal tissues ($p < 0.01$, Figure 1).

Correlation of DICER1-AS1 Expression with Clinicopathological Features

To investigate the relationship between DICER1-AS1 expression and clinical features in osteosarcoma, all osteosarcoma patients were classified into two groups depending on DICER1-AS1 levels in tumor tissues relative to the median

Table I. Primer sets used in the present study.

Gene names	Forward (5′-3′)	Reverse (5′-3′)	Application
DICER1-AS1	TGACCAGTCTTACCCCTCCT	CTGAAGCACCTGAAATGCG	Real-time RT-PCR
U6	ACCCTGAGAAATACCCTCACAT	GACGACTGAGCCCCTGATG	Real-time RT-PCR

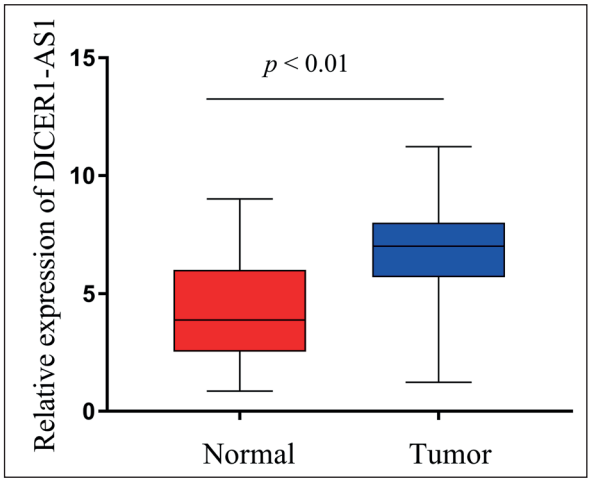

Figure 1. Expression of DICER1-AS1 in osteosarcoma tissues and matched normal bone tissues. DICER1-AS1 expression was significantly higher in osteosarcoma tissues than in the matched normal bone tissues ($p < 0.01$).

ratio: high DICER1-AS1 expression group (n = 110) and low DICER1-AS1 expression group (n = 104). As shown in Table II, it was observed that DICER1-AS1 expression was closely associated with clinical stage ($p = 0.005$) and distant metastasis ($p = 0.000$). However, there were no relationships between DICER1-AS1 expression and other clinicopathological parameters, including age, gender, tumor size, anatomic location and response to chemotherapy (all $p > 0.05$).

Association of DICER1-AS1 Expression with Prognosis of Osteosarcoma Patients

In order to further explore the prognostic value of DICER1-AS1 expression in osteosarcoma, survival curves were constructed by Kaplan-Meier method and compared by the log-rank test. As shown in Figure 2, osteosarcoma patients with higher DICER1-AS1 expression level had shorter OS than those with high DICER1-AS1 expression level ($p = 0.007$). Similarly, we also found that DICER1-AS1 expression was significantly correlated with osteosarcoma patients' DFS ($p < 0.0001$, Figure 3). In the multivariate analysis using the Cox proportional hazards model, we further found that DICER1-AS1 expression ($p = 0.004$ and 0.001, respectively), clinical stage ($p = 0.015$ and 0.004, respectively) and distant metastasis ($p = 0.006$ and 0.001, respectively) were independent prognostic factors for both OS and DFS in osteosarcoma patients (Table III).

Discussion

Despite the advances in therapeutic strategies, outcome remains poor for most osteosarcoma patients with metastatic or recurrent osteosarcoma; therefore, understanding the mechanisms underlying osteosarcoma pathogenesis may help yield novel biomarkers for early detection, prog-

Table II. Association of DICER1-AS1 expression with clinicopathologic features of osteosarcoma.

Clinicopathologic features	No. of cases	DICER1-AS1 expression		*p*-value
		High	Low	
Age (y)				0.565
< 25	100	48	52	
≥ 25	114	62	52	
Gender				NS
Male	136	70	66	
Female	78	40	38	
Tumor size (cm)				NS
> 8	106	60	46	
≥ 8	108	50	58	
Anatomic location				NS
Tibia/femur	135	65	70	
Elsewhere	79	45	34	
Response to chemotherapy				NS
Good	116	55	61	
Poor	98	55	43	
Clinical stage				0.005
IIA	134	59	75	
IIB/III	80	51	29	
Distant metastasis				0.000
Absent	145	64	81	
Present	69	46	23	

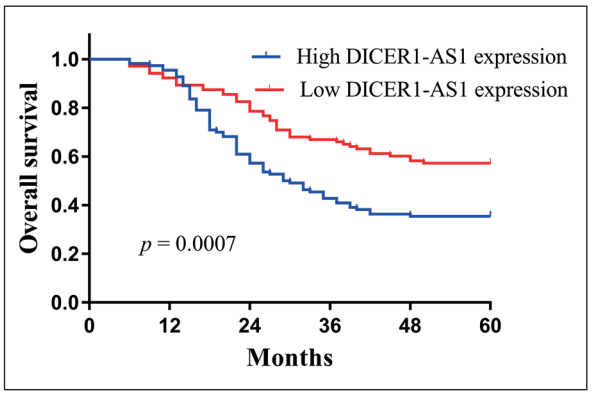

Figure 2. Kaplan-Meier postoperative survival curve for patterns of patients with osteosarcoma. Osteosarcoma patients with high DICER1-AS1 expression showed shorter overall survival time than those with low DICER1-AS1 expression ($p = 0.0007$).

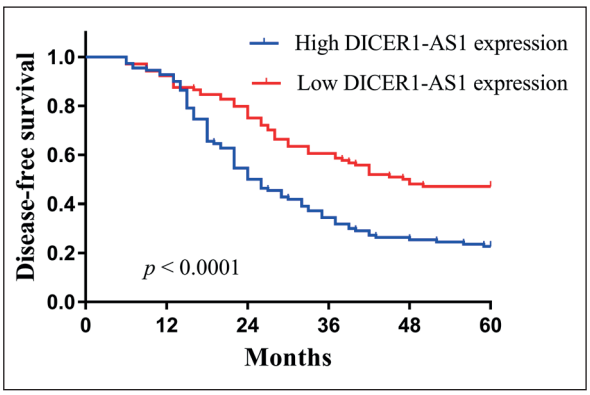

Figure 3. Kaplan-Meier postoperative survival curve for patterns of patients with osteosarcoma. Osteosarcoma patients with high DICER1-AS1 expression showed shorter disease-free survival time than those with low DICER1-AS1 expression ($p < 0.0001$).

nosis and treatment[17,18]. Numerous studies[19-21] have demonstrated that several lncRNAs, such as lncRNA TP73-AS1, lncRNA ZFAS1, lncRNA XIST, may serve as a diagnostic and prognostic marker in various tumors, including osteosarcoma. In the present study, our data indicated that DICER1-AS1 was important for osteosarcoma initiation and progression and held promise as a prognostic biomarker to predict survival in osteosarcoma. Emerging studies have confirmed that lncRNAs are involved in cell proliferation, apoptosis, invasion and metastasis in various types of cancers, including osteosarcoma[22]. For instance, Zhang et al[23] reported that lncRNA FOXC2-AS1 was highly expressed in osteosarcoma tissues and cell lines and associated with poor prognosis of osteosarcoma patients, and its forced expression promoted doxorubicin resistance in osteosarcoma by modulating FOXC2 expression. Cui et al[24] found that the expression of lncRNA HOXA11-AS was significantly up-regulated in osteosarcoma patients and associated with advanced clinical stage, distant metastasis and poor overall survival of osteosarcoma. Further gain- and lost-of-function assay showed that HOXA11-AS served as a tumor promoter by sponging miR-124-3p in osteosarcoma. Wen et al[25] reported that lncRNA UCA1 have the potential to be a promising biomarker for discriminating patients with osteosarcoma from healthy controls and predicting the prognosis of osteosarcoma patients. Recently, Gu et al[16] firstly reported DICER1-AS1 as a up-regulated lncRNA in osteosarcoma cells. Then, they performed *in vivo* and *in vitro* assay, finding that knockdown of DICER1-AS1 significantly suppressed the proliferation, invasion and autophagy of osteosarcoma cells via miR-30b/ATG5. However, whether DICER1-AS1 was up-regulated in clinical osteosarcoma tissues, and its prognostic value have not been investigated. In this study, based on the results of DICER1-AS1 expression in osteosarcoma cells, we firstly performed

Table III. Multivariate survival analysis of overall survival and disease-free survival in 214 osteosarcoma patients.

Variables	Overall survival			Disease-free survival		
	RR	95% CI	p	RR	95% CI	p
Age	1.562	0.782-2.233	0.415	1.493	0.944-2.039	0.327
Gender	1.836	0.559-2.672	0.232	1.553	0.739-2.328	2.118
Tumor size	2.214	0.932-2.873	0.118	1.884	0.582-2.449	0.217
Anatomic location	1.664	0.873-2.326	0.177	1.885	0.942-2.683	0.114
Response to chemotherapy	1.347	0.739-2.215	0.114	1.573	0.949-2.443	0.134
Clinical stage	3.149	1.033-4.237	0.015	3.673	1.235-5.623	0.004
Distant metastasis	3.136	1.235-5.028	0.006	4.237	1.663-6.238	0.001
DICER1-AS1 expression	3.236	1.148-5.347	0.004	3.935	1.556-6.349	0.001

RT-PCR to detect the levels of DICER1-AS1 in osteosarcoma patients. Consistent with previous study, our results also confirmed DICER1-AS1 as an up-regulated lncRNA in osteosarcoma patients. Subsequently, clinical assay revealed that DICER1-AS1 expression was closely associated with clinical stage and distant metastasis, indicating that DICER1-AS1 may contribute to malignant tumor. Moreover, based on Kaplan-Meier survival analysis, we demonstrated that higher DICER1-AS1 expression was associated with poorer DFS and OS, when compared to lower DICER1-AS1 expression. Most importantly, multivariate analysis revealed that DICER1-AS1 could be used as an independent prognostic factor for both OS and DFS in osteosarcoma patients. Our finding, for the first time, indicated the possibility of using high expression levels of ICER1-AS1 as a predictor for prognosis and survival. However, to be honest, one limitation of the current study is that there was relatively small sample size enrolled. Thus, larger sample size will be needed to further demonstrate our findings.

Conclusions

We suggest that high levels of DICER1-AS1 might reflect a less aggressive osteosarcoma phenotype and predict better survival in patients with osteosarcoma. DICER1-AS1expression may be a useful prognostic marker for this disease.

Conflict of Interest

The Authors declare that they have no conflict of interests.

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