

Analysis of circulating long non-coding RNA UCA1 as potential biomarkers for diagnosis and prognosis of osteosarcoma

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Abstract. – OBJECTIVE: The aim of this investigation was to examine the potential usefulness of long non-coding RNA UCA1 (UCA1) as a biomarker for diagnosis and prognosis in osteosarcoma.

PATIENTS AND METHODS: The expression level of UCA1 was determined using TaqMan real-time PCR in human osteosarcoma tissues and patients' sera. Next, we investigated to clarify the relationship of UCA1 with clinicopathological features. The receiver operating characteristic (ROC) curve was performed to estimate the diagnostic value of UCA1. Finally, the prognosis of patients with osteosarcoma was assessed by Kaplan-Meier method and Cox proportional hazards model.

RESULTS: We observed that UCA1 was significantly increased in osteosarcoma tissue compared with normal bone tissue ($p < 0.001$) and the serum expression of UCA1 was significantly higher in patients with osteosarcoma than that in healthy controls ($p < 0.01$). Up-regulation of UCA1 was correlated with clinical stage ($p = 0.001$) and metastasis ($p = 0.007$). Furthermore, serum UCA1 levels were observed to be robust in differentiating osteosarcoma patients from control subjects [area under the curve (AUC) = 0.831; 95% confidence interval (CI) = 0.746 to 0.916]. Kaplan-Meier analysis showed that high UCA1 expression level was associated with poorer overall survival ($p < 0.001$) and disease-free survival ($p < 0.001$). Finally, Cox regression analyses showed that UCA1 expression might be an independent prognostic parameter to predict poor prognosis.

CONCLUSIONS: Our study firstly showed that UCA1 could be a specific and noninvasive candidate biomarker for the diagnosis and prognosis of UCA1.

Key Words:

Long non-coding RNA, UCA1, Osteosarcoma, Prognosis, Diagnosis.

Introduction

Osteosarcoma is a common malignant bone tumor and mainly occurs in adolescents and young adults worldwide¹. The most frequently affected joint is the knee, when tumors arise from the distal femur and proximal tibia². With the development of osteosarcoma therapy such as wide tumor excision, radiotherapy, and adjuvant chemotherapy, five-year overall survival rates have been reported as 78%^{3,4}. But overall survivals are usually lower for non-responders to chemotherapy and patients with metastasis⁵. Therefore, to develop new non-invasive and early diagnostic methods are highly needed.

Long noncoding RNA (lncRNA) is transcribed RNA molecules more than 200 nucleotides and lack protein-coding potential⁶. They are considered as useless "dark matter" in gene expression for decades. However, more and more studies revealed that lncRNAs play crucial roles in multiple biological processes such as development, differentiation, and carcinogenesis by through regulating gene expression^{7,8}. Furthermore, various regulation mechanisms have been identified in various tumors⁹. For instance, Cai et al¹⁰ showed that overexpression of long noncoding RNA CCAT2 could promote breast tumor growth by regulating the Wnt signaling pathway. Sun et al¹¹ showed that long non-coding RNA NEAT1 promoted NSCLC cell growth and metastasis by acting as a ceRNA. Those results revealed that lncRNA might serve as important potential biomarkers in tumors. Thus, it provided the support for lncRNA to be used as an indicator for the diagnosis and prognosis of tumors.

lncRNA UCA1 was originally identified in human bladder carcinoma¹². Then, the abnormal expression of UCA1 was found in several tumors^{13,14}. However, to our best knowledge, the diagnostic and prognostic values of UCA1 in osteosarcoma have not been reported. In the present study, we investigated the UCA1 expression in osteosarcoma tissues and patients' sera, and then we explored the relationship between expression of UCA1 in osteosarcoma tissues and the clinicopathological parameters. Furthermore, we performed ROC and multivariate analysis to determine its diagnostic and prognostic values in osteosarcoma. Our findings suggested that UCA1 may serve as a prognostic and diagnostic marker.

Patients and Methods

Patients and Tissue Samples

This study was approved by the Ethical Review Committee of Second Laiyang Central Hospital, Yantai, Shandong, China. All patients enrolled in the current study gave their written informed consent. Tumor tissues and noncancerous bone tissues from 151 osteosarcoma patients were retrospectively selected from the surgical pathology records of Laiyang Central Hospital from 2008 to 2010. None of the patients had received radiotherapy and chemotherapy before the tissues were collected. These tissue samples were

frozen in liquid nitrogen and kept at -80°C until RNA extraction. Serum specimens were from 85 osteosarcomas and 74 healthy controls. All the patients were followed up every month by telephone until death or the end of follow-up. The clinicopathological information of the patients is shown in Table I.

RNA Extraction and Quantitative Real-time PCR

Total RNA was extracted from osteosarcoma tissues and sera samples using the TRIzol reagent (Qiagen, Inc., Valencia, CA, USA). CDNA was synthesized from total RNA with oligo (dT) primers by using the Omniscript cDNA Kit (GenePharma, Pudong, Shanghai, China). RT and qPCR kits were used to evaluate the expression of UCA1 in the osteosarcoma tissues and sera samples. The primers used in this work were UCA1, 5'-TTCCTTATTATCTCTTCTG-3' (forward) and 5'-TCCATCATACGAATAGTA-3' (reverse); GAPDH, 5'-CTCGCTTTGGCAGCACA-3' (forward) and 5'-AACGCTTCACGAATTTGCGT-3' (reverse).

GAPDH were used internal controls for the expression of UCA1. The Ct value of UCA1 was quantified with the 2- $\Delta\Delta$ Ct method.

Statistical Analysis

Statistical analyses were performed using SPSS Statistics 17.0 software (SPSS Inc., Chicago, IL, USA). χ^2 -test and Fisher's exact method were applied for assessing the relationship between UCA1 expression and patients' clinicopathological parameters. Receiver-operating characteristic (ROC) curves were used to evaluate the performance of UCA1 to discriminate osteosarcoma patients from controls. Survival curves were calculated by the Kaplan-Meier method and compared by the log-rank test. Cox's proportional hazards model was used to determine the independent association of prognostic variables with DFS and OS. *p*-value of < 0.05 was considered statistically significant.

Results

High Expression of UCA1 in Osteosarcoma Tissues and Patients' Sera

Real-time quantitative RT-PCR was performed to detect the expression levels of UCA1 in 151 pairs of osteosarcoma and adjacent normal bone

Table I. Correlations between circulating lncRNA UCA1 and clinicopathological variables.

Parameters	No. of cases	UCA1-high (no.)	UCA1-low (no.)	<i>p</i> -value
Gender				0.572
Male	92	44	48	
Female	59	31	28	
Age				0.199
≤18	95	51	44	
>18	56	24	32	
Tumor site				0.804
Femur/Tibia	79	40	39	
Others	72	35	37	
Tumor size				0.907
≤ 8 cm	63	31	32	
>8 cm	88	44	44	
Clinical stage				0.001
IIA	61	20	41	
IIB/III	90	55	35	
Metastasis				0.007
Absent	79	31	48	
Present	72	44	28	

Table II. Multivariate analysis of 5-year overall and disease-free survival in patients with osteosarcoma.

Variable	Overall survival			Disease-free survival		
	HR	95% CI	p-value	HR	95% CI	p-value
Gender	1.652	0.823-2.231	0.295	1.955	1.137-2.884	0.265
Age	2.147	1.284-3.554	0.392	2.663	1.613-3.227	0.417
Tumor site	2.766	1.652-3.885	0.514	2.994	1.932-3.427	0.367
Tumor size	2.231	1.023-3.354	0.221	2.477	1.223-3.764	0.265
Clinical stage	3.357	1.146-5.523	0.009	2.964	1.317-5.944	0.006
Metastasis	2.881	1.147-5.893	0.003	2.145	0.893-5.133	0.006
UCA1 expression	2.516	1.345-4.834	0.011	3.137	1.664-6.159	0.003

tissues. As shown in Figure 1A, UCA1 was up-regulated in osteosarcoma tissues compared with normal ones ($p < 0.01$). Next, we further explore the expression levels of UCA1 in osteosarcoma patients' sera and healthy controls. Our results showed that expression levels of UCA1 in the plasma of osteosarcoma patients were significantly higher compared with controls ($p < 0.01$, Figure 1B). Our findings revealed that UCA1 might be involved in the progression of osteosarcoma.

Association Between UCA1 Expression and Clinicopathological Features

To explore whether UCA1 was associated with the progression of osteosarcoma, we investigated the relationship between its expression with clinical features. As shown in Table I, the expression of UCA1 was significantly correlated with clinical stage and metastasis ($p < 0.05$). However, no

statistically significant correlations were identified between the expression of UCA1 and other clinicopathologic characteristics including age, gender, tumor site and tumor size. These results suggest that UCA1 associated to the aggressiveness of osteosarcoma patients.

Validation of Circulating UCA1 in Diagnosing Osteosarcoma

To determine the diagnostic value of UCA1 for osteosarcoma, we generated receiver operating characteristic (ROC) curves. Figure 2 showed that the predictive performance of circulating UCA1 in distinguishing patients with osteosarcoma from healthy controls, the AUC was 0.831 (95% CI, 0.746-0.916). Our results suggested that UCA1 might be an unreliable biomarker for osteosarcoma and provide a new tumor marker for the diagnosis of osteosarcoma.

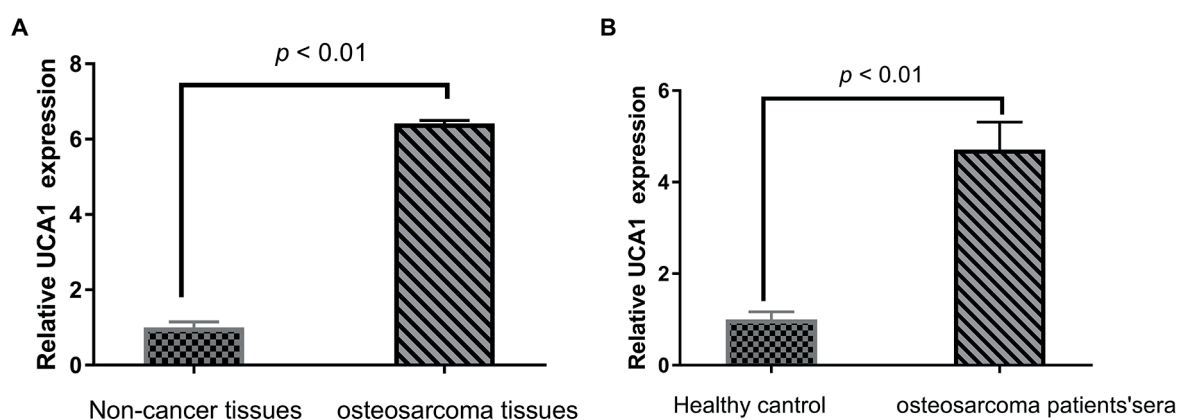


Figure 1. Expression of UCA1 was significantly upregulated in osteosarcoma tissues and patients' sera. (A) The expression level of UCA1 in osteosarcoma patients (B) The expression level of plasma UCA1 in osteosarcoma patients and healthy controls.

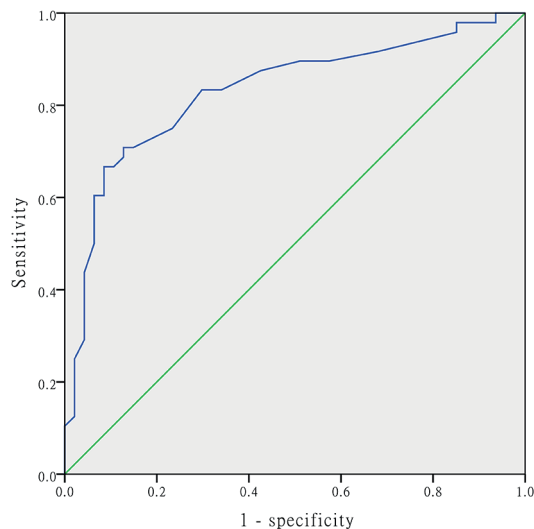


Figure 2. ROC curve for diagnostic value of serum UCA1 in osteosarcoma.

UCA1 Predicted Poor Prognosis of Osteosarcoma Patients

To further verify the potential clinical utility of the UCA1 expression, we performed Kaplan-Meier analysis and log-rank test to explore the association between UCA1 expression and survival of osteosarcoma patients. 151 patients were divided into the UCA1 high group ($n = 75$) and the UCA1 low group ($n = 76$) according to their relative UCA1 expression. As shown in Figure 3 and Figure 4, the high UCA1 expression group also exhibited a significantly lower 5-year OS and DFS than the low UCA1 expression group ($p < 0.001$, respectively). Finally, we performed multivariate analysis to determine whether UCA1 expression could be an independent prognostic factor. As shown in Table II, the results indicated that UCA1 was an independent prognostic factor for OS and DFS for osteosarcoma patients.

Discussion

Osteosarcoma remains to be the third highest cause of cancer-related death in children and young adults¹⁵, so exploring new therapies, clinical biomarkers and treatment targets for treatment of osteosarcoma is important for improving the clinical outcome. Many studies have been conducted to search for robust tumor markers, but the results were not very satisfying. An increasing number of evidence showed that

lncRNAs play a key role in the development and progression of osteosarcoma and could be employed to develop as biomarkers and prognosis factors. In the present work, we focused on lncRNA UCA1.

Previous research has shown that UCA1 was involved in the progression of various tumors. For instance, Xiao et al¹⁶ showed that UCA1 promoted cell migration and invasion via enhancing Wnt/beta-catenin signaling pathway. Yang et al¹⁷ observed that UCA1 acted as a competing endogenous RNA to promote ovarian cancer cells growth and metastasis by sponging miR-485-5p. Wang et al¹⁸ reported that UCA1 could promote malignant progression of human hepatocellular carcinoma through inhibition of miR-216b and activation of the FGFR1/ERK signaling pathway. All these findings revealed that UCA1 served as a tumor promoter in different tumors. Also, UCA1 was reported to correlate with poor prognosis in lung cancer¹⁹ and esophageal squamous cell carcinoma²⁰. Thus, we wondered if UCA1 could be used as a potential biomarker for the diagnosis and prognosis of osteosarcoma. Most recently, Li et al²¹ reported that UCA1 expression was upregulated in osteosarcoma, and over-expression of UCA1 promoted cell proliferation and metastasis. This finding supports our opinion.

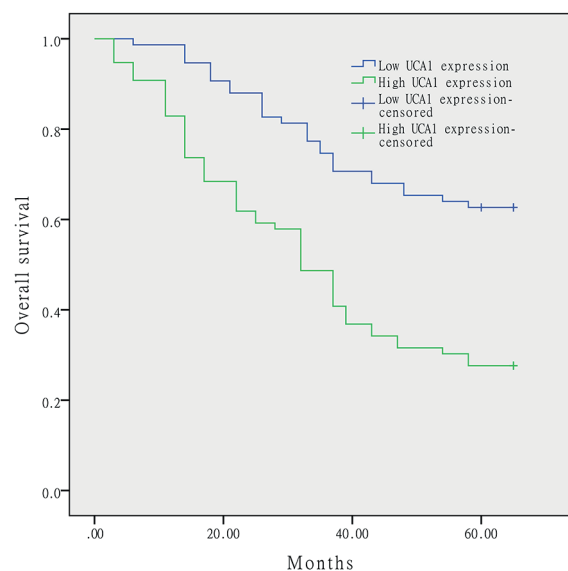


Figure 3. Kaplan-Meier curves of the overall survival (OS) of 151 osteosarcoma patients. The group with high UCA1 expression exhibited a significantly shorter OS compared with the group with low UCA1 expression ($p < 0.001$).

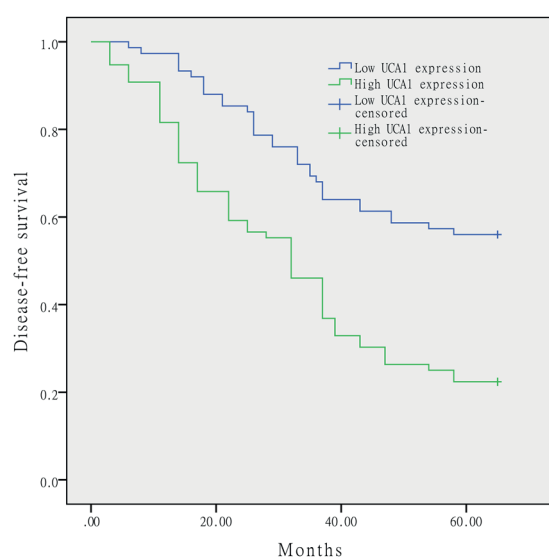


Figure 4. Kaplan-Meier curves of the disease-free survival (DFS) of 151 osteosarcoma patients. The group with high UCA1 expression exhibited a significantly shorter DFS compared with the group with low UCA1 expression ($p < 0.001$).

In the present report, we found that the UCA1 levels in serum samples from osteosarcoma patients were significantly higher than those in healthy controls. Furthermore, we found that up-regulation of UCA1 was correlated with clinical stage and metastasis. ROC curve analysis showed that UCA1 was an useful marker for discriminating cases from healthy controls, with an AUC of 0.831. We also found that UCA1 expression positively correlated with OS and DFS in osteosarcoma patients. Osteosarcoma patients with downregulated lncRNA-ATB levels had longer OS and DFS times. Finally, we performed Cox regression analyses to determine the prognostic values of UCA1 in osteosarcoma. The results showed that UCA1 was independent poor prognostic factors.

Conclusions

Our present data revealed that UCA1 might serve as novel noninvasive biomarkers to distinguish patients with benign and osteosarcoma, as well as help us judge the progression of osteosarcoma. Further studies are needed for their diagnostic and prognostic value using a larger cohort.

Conflict of interest

The authors declare no conflicts of interest.

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