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Research paper

Overexpression of lncRNA UCA1 promotes osteosarcoma progression and correlates with poor prognosis

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ABSTRACT

Long non-coding RNAs (lncRNAs) have been proved to play important roles in the tumorigenesis and development of several human malignancies. Our study aims to investigate the expression and function of lncRNA-UCA1 in osteosarcoma. lncRNA-UCA1 expression was detected in osteosarcoma tissues and cell lines by using qRT-PCR. Association between lncRNA-UCA1 levels and clinicopathological factors and patient's prognosis was analyzed. The roles of lncRNA-UCA1 in regulating osteosarcoma cell proliferation, apoptosis, migration, and invasion were evaluated in vitro. We found that lncRNA-UCA1 expression was upregulated in osteosarcoma tissues and cell lines. High lncRNA-UCA1 expression was significantly correlated with large tumor size, high tumor grade, positive distant metastasis, and advanced clinical stage. Multivariate regression analysis identified lncRNA-UCA1 overexpression as an independent unfavorable prognostic factor. lncRNA-UCA1 knockdown inhibited osteosarcoma cell proliferation, promoted cell apoptosis, and suppressed cell invasion and migration, whereas lncRNA-UCA1 overexpression showed opposite effects. These findings suggested that lncRNA-UCA1 may contribute to osteosarcoma initiation and progression, and would be not only a novel prognostic marker but also a potential therapeutic target for this disease.

1. Introduction

Osteosarcoma is one of the most common primary bone tumor and occurs predominantly in children and young adults [1]. With the development of multiple therapeutic strategies including wide tumor excision, neoadjuvant or adjuvant chemotherapy, and radiotherapy, the 5 year survival of the non-metastatic patients has increased to 65% [2]. However, osteosarcoma is very aggressive and approximately 40–50% of patients will eventually develop metastases, especially pulmonary metastases [3]. The prognosis of these patients is rather poor, and the long-term survival rate is only 10–30% [4]. The complex molecular mechanisms underlying osteosarcoma tumorigenesis and progression remain largely unclear. Identification of new candidate molecules that take part in these processes is crucial for developing new therapeutic approach for osteosarcoma and improving clinical outcomes of patients with this disease.

Long noncoding RNAs (lncRNAs) are defined as RNA transcripts of more than 200 nucleotides in length with no or little protein-coding capacity [5]. LncRNAs can regulate gene expression through diverse mechanisms including epigenetic silencing, mRNA splicing, lncRNA-miRNA interaction, lncRNA-protein interaction and lncRNA-mRNA interaction [6]. Recent studies showed that lncRNAs are involved in a wide range of biological processes, such as embryonic development, cell proliferation, apoptosis, invasion, metastasis and angiogenesis [7–9]. Furthermore, lncRNAs can act as oncogenes or tumor suppressors, and play important roles in carcinogenesis and cancer development [10,11]. Abnormal lncRNA expression and its association with various important clinicopathological parameters have been reported in many types of cancers. In terms of osteosarcoma, increased lncRNA FGFR3-AS1 expression correlated with large tumor size, advanced Enneking stage, and poor survival [12]. Plasma lncRNA TUG1 contributed to osteosarcoma detection and dynamic surveillance [13]. Silence of lncRNA TUSC7 promoted osteosarcoma cell proliferation and increased colony formation in vitro [14]. LncRNA ODRUL inhibition could inhibit osteosarcoma cell proliferation and migration, and partly reversed doxorubicin resistance [15]. Therefore, lncRNAs may be utilized for osteosarcoma diagnosis and prognosis, and serve as potential therapeutic targets.

Abbreviations: lncRNA, long noncoding RNA; UCA1, Urothelial carcinoma associated 1; RT-PCR, reverse transcription-polymerase chain reaction; DMSO, dimethyl sulfoxide; MTT, 3-(4,5-dimethylthiazol-2-yl)−2,5-diphenyltetrazolium bromide; DMEM, Dulbecco's modified Eagle's medium; TNM, tumor-node-metastasis

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Urothelial carcinoma associated 1 (UCA1) is an lncRNA originally identified in human bladder carcinoma [16]. Overexpression of UCA1 significantly enhanced bladder cancer cell proliferation and migration and confered drug resistance. Recently, UCA1 has been reported to be upregulated and exert its oncogenic activity in several cancers such as esophageal squamous cell carcinoma [17], breast cancer [18], non-small cell lung cancer [19], gastric cancer [20], colorectal cancer[21], hepatocellular carcinoma [22], renal cell carcinoma [23], ovarian cancer [24], and prostate cancer [25]. However, the significance of UCA1 in osteosarcoma is still unclear. In the present study, we investigated the expression level of UCA1 in osteosarcoma samples and cell lines. We also investigated the correlation between UCA1 expression and clinicopathological characteristics and patient's survival. Moreover, we explored the role of UCA1 in the regulation of biological behaviors of osteosarcoma cells.

2. Materials and methods

2.1. Patients and clinical specimens

Matched fresh osteosarcoma specimens and adjacent non-tumorous tissues were acquired from 135 patients at Hanzhong Municipal Central Hospital between January 2006 and December 2010. None of the patients received chemotherapy or radiotherapy before sample collection. All specimens were frozen in liquid nitrogen immediately after collection and stored at −80 °C until use. Table 1 showed the details of clinical and pathological characteristics of the patients. The follow-up data were available and complete for each patient. Overall survival was calculated from the day of primary surgery to death or last follow-up. This project was approved by the Clinical Research Ethics Committee of our hospital and all patients provided written informed consent.

2.2. Cell culture

The normal osteoblast cell line hFOB1.19 and human osteosarcoma cell lines (HOS, Saos-2, MG-63, U2OS) were purchased from the Chinese Academy of Medical Sciences and cultured in Dulbecco's modified Eagle's medium (DMEM, USA) supplemented with 10% fetal bovine serum (FBS, USA), penicillin (100 U/ml), and streptomycin (100 μg/ml). All cells were incubated at 37 °C in 5% CO_2.

2.3. RNA extraction and real-time PCR

Total RNA was extracted by Trizol reagent (Invitrogen, Carlsbad, CA, USA). The first-strand cDNA was synthesized from 1 μg of total RNA using the Reverse Transcription System Kit (Takara, Dalian, China). The real-time PCR was carried out using SYBR Premix Ex Taq kit (Takara, Dalian, China) on an ABI 7500 Real-Time PCR System (Applied Biosystems, CA, USA). Each assay was performed in triplicate, and GAPDH was used as the endogenous control gene. The primer sequences used were as follows: UCA1, 5′-TTCCTTATTATCTCTTCTG-3′ (forward) and 5′-TCCATCATACGAATAGTA-3′ (reverse); GAPDH, 5′-CTCGCTTTGGCAGCA CA-3′ (forward) and 5′-AACGCTTCACGAATTTGCGT-3′ (reverse). The relative amount of UCA1 to GAPDH was calculated using the $2^{-\Delta Ct}$ method, where $\Delta CT = (CT_{UCA1} - CT_{GAPDH})$.

2.4. UCA1 knockdown and overexpression

The cDNA encoding UCA1 was PCR-amplified and subcloned into the pCDNA3.1 vector (Invitrogen, Shanghai, China). The empty pcDNA3.1 vector was used as the control. UCA1 small interfering RNA (si-UCA1) and negative control siRNA (si-NC) were synthesized by GenePharma (Shanghai, China). Transfections were performed using Lipofectamine 2000 according to the manufacturer's instructions (Invitrogen, CA, USA).

Table 1
Association of lncRNA-UCA1 expression with clinicopathological factors in osteosarcoma.

Clinicopathological features	Number of cases	lncRNA-UCA1 expression		P
		Low n (%)	High n (%)	
Age				
< 20 years	98	46(46.9%)	52(53.1%)	0.339
≥ 20 years	37	21(56.8%)	16(43.2%)	
Gender				
Male	95	49(51.6%)	46(48.4%)	0.573
Female	40	18(45.0%)	22(55.0%)	
Tumor size				
> 8 cm	57	20(35.1%)	37(64.9%)	0.005
≤ 8 cm	78	47(60.3%)	31(39.7%)	
Anatomic location				
Tibia/femur	93	48(51.6%)	45(48.4%)	0.512
Elsewhere	42	19(45.2%)	23(54.8%)	
Tumor grade				
Low	45	30(66.7%)	15(33.3%)	0.004
High	90	37(41.1%)	53(58.9%)	
Histological type				
Osteoblastic	52	28(53.8%)	24(46.2%)	0.774
Fibroblastic	26	11(42.3%)	15(57.7%)	
Chondroblastic	20	9(45.0%)	11(55.0%)	
Telangiectatic	21	12(57.1%)	9(42.9%)	
Others	16	7(43.8%)	9(56.2%)	
Enneking stage				
I	35	27(77.1%)	8(22.9%)	< 0.001
II	66	32(48.5%)	34(51.5%)	
III	34	8(23.5%)	26(76.5%)	
Distant metastasis				
Absent	101	58(57.4%)	43(42.6%)	0.002
Present	34	9(26.5%)	25(73.5%)	

2.5. Cell proliferation assay

Osteosarcoma cells were seeded into 96-well plates after transfection. Cell density was adjusted to 5×10^3/well, and the final volume was 150 μl/well. Cell proliferation was determined every 24 h for 4 days. At the indicated time point, 20 μl of MTT (Sigma, USA) was added into each well and the cells were cultured for another 4 h at 37 °C. Then the supernatants were removed and 150 μl of DMSO was added. Optical density was detected at a wavelength of 490 nm and each assay was repeated three times.

2.6. Detection of apoptosis by flow cytometry

Cell apoptosis was measured by using an Annexin V-FITC Apoptosis Detection kit (BD Biosciences, San Diego, CA) according to the manufacturer's instructions. After transfection, osteosarcoma cells were harvested and washed twice with cold phosphate-buffered saline. Then, the cells were treated with Annexin V/