Expression level of *CDC2* gene in osteosarcoma and its clinical significance

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Abstract. The aim of the present study was to investigate the expression of cell division cycle gene 2 (*CDC2*) in osteosarcoma tissues and its clinical significance. Specimens of cancer tissues, paracancerous tissues and serum from 47 patients hospitalized at the Department of Orthopedics at The Third Affiliated Hospital of Sun Yat-sen University (Guangzhou, China) from January, 2010 to January, 2015 and serum from 35 normal subjects were collected. The expression of CDC2 mRNA was evaluated using quantitative polymerase chain reaction (RT-PCR) and the relationship between CDC2 protein expression and clinical features of patients with osteosarcoma was analyzed. There was a significant difference in the expression levels of CDC2 between cancer (2.31±0.306) and paracancerous tissues (0.91±0.251) (P<0.05), and there was a difference in the expression of CDC2 in serum between patients (1.58±0.149) and the normal control group (0.67±0.136). Receiver operating characteristic (ROC) curve analysis indicated that CDC2 was of great value in the diagnosis of osteosarcoma. The expression of CDC2 was closely related to the tumor diameter (P<0.05), World Health Organization classification (P<0.05) and KPS score (P<0.05). However, there was no significant association between the expression of CDC2 and factors including age and sex (both P>0.05). The high expression of CDC2 was closely related to the lower survival rate in patients with osteosarcoma (P<0.05). The increase of the tumor-node-metastasis (TNM) staging of osteosarcoma and the high expression of CDC2 are the risk factors affecting the prognosis of osteosarcoma patients (P<0.05), and Cox regression analysis showed that the expression level of CDC2 was a risk factor affecting the prognosis of osteosarcoma patients (P<0.05). The results indicate that CDC2 is highly expressed in osteosarcoma and may be a biomarker to predict the occurrence, development and prognosis of osteosarcoma.

Introduction

Osteosarcoma is a very common osteoblast malignant tumor, which is prone to metastasis, especially lung tissue metastasis. It is highly malignant and the prognosis is not satisfactory (1,2). Two to three out of one million individuals suffer from osteosarcoma each year, mainly male minors.

The treatment methods are becoming increasingly advanced and the prognosis of the patient has improved obviously since the 1870s (3). The currently used comprehensive treatment mode is preoperative chemotherapy-surgery-postoperative adjuvant chemotherapy and the highest 5-year survival rate of the patients has reached 80%. Nevertheless, there are patients who die after treatment failure (4). More research is needed to further understand the occurrence and the development of osteosarcoma, the pathogenesis of osteosarcoma, the development of the disease and the transfer mechanism to identify an ideal therapeutic target. The human cell division cycle gene 2 (*CDC2*), and its encoded cyclin CDC2 protein participates in regulating the transition of phase G2 into phase M in the interphase of mitosis (5,6).

The pathogenesis and progression of cancer are related to the abnormal regulation of the cell cycle, and there is a high expression of CDC2 in many malignant tumors (7-18). However, there is less research on the expression level and clinical significance of CDC2 in osteosarcoma (19). In the present study, quantitative polymerase chain reaction (RT-PCR) was used to detect the expression of CDC2 in order to explore its clinical significance.

Materials and methods

Clinical data. Specimens of cancer, paracancerous tissues and serum from 47 patients hospitalized at the Department of Orthopedics at The Third Affiliated Hospital of Sun Yat-sen University (Guangzhou, China) from January, 2010 to January, 2015 and serum of 35 normal subjects were collected. The expression of *CDC2* was detected via PCR and the relationship between CDC2 expression and clinical features of patients with osteosarcoma was analyzed. The instruments and reagents used in this study are shown in Table I. The study was approved

Table I. Instruments and reagents.

Instruments and reagents	Manufacturer
PCR instrument	Applied Biosystems; Thermo Fisher Scientific, Inc. (Waltham, MA, USA)
Spectrophotometer SMA5000	Merinton Instrument, Inc. (Ann Arbor, MI, USA)
Reverse transcription kit	Fermentas; Thermo Fisher Scientific, Inc.
U6 internal reference	Guangzhou Shangeng Biological Technology Co., Ltd. (Guangzhou, China)
100 bp DNA Marker	Tiangen Biotech Co., Ltd. (Beijing, China)
2X Taq PCR MasterMix	Tiangen Biotech Co., Ltd.
TRIzol	Tiangen Biotech Co., Ltd.
DEPC	Sigma Sigma-Aldrich; Merck KGaA (Darmstadt, Germany)

PCR, polymerase chain reaction; DEPC, diethyl pyrocarbonate.

Table II. Primer sequences.

Primer	U6 internal reference	CDC2
F	5'-CTCGCTTCGGCAGCACA-3	5'-TACCTATGGAGTTGTGTATAA-3'
R	5'-AACGCTTCACGAATTTGCGT-3'	5''-ATTCCACTTCTGGCCACACTT-3'

F, forward; R, reverse; CDC2, cell division cycle gene 2.

by the Ethics Committee of The Third Affiliated Hospital of Sun Yat-sen University, and the patient or their families signed informed consent.

Detection of the CDC2 expression via RT-PCR. CDC2 was extracted from tissues and serum in strict accordance with the instructions provided by Sigma-Aldrich (St. Louis, MO, USA) and Merck KGaA (Darmstadt, Germany), respectively, and the purity of RNA was expressed by the ratio of the absorbance value from 260 to 280 nm. Purity was satisfactory if the result was between 1.9 and 2.1; otherwise, the purification was repeated until it was up to the standard.

RT-PCR. The experiment was conducted in strict accordance with the instructions of reverse transcription kits (Fermentas; Thermo Fisher Scientific, Inc., Waltham, MA, USA). The PCR reaction system was measured as: 25 µl, *CDC2* annealing at 53°C, 25 cycles. Primer sequences are shown in Table II. For the statistical analysis, three parallel wells were set for all samples, and the average was taken. With U6 as the internal reference, the relative expression level of CDC2 was expressed as $2^{-\Delta\Delta Cq}$.

Statistical analysis. SPSS 22.0 (IBM Corp., Armonk, NY, USA) was used for data analysis. The post hoc test was SNK test. Measurement data are presented as mean ± SD, and the analysis of variance was used for the comparison among groups. The t-test was used for the comparisons of CDC2 expression levels in specimens of cancer, paracancerous tissues and serum and the serum of 35 normal subjects, and the Chi-square test was used for the comparison of parametres including sex and age. The receiver operating characteristic (ROC) curve was drawn to assess the diagnosis value of serum CDC2 in patients

with osteosarcoma and the relationship between CDC2 and osteosarcoma was analyzed via univariate and multivariate Cox regression analysis. $P < 0.05$ was considered to indicate a statistically significant difference.

Results

Expression levels of CDC2 in tissues, cells and blood. CDC2 was highly expressed in cancer tissues, which was higher than that in paracancerous tissues ($P < 0.05$). The expression level in the blood of patients was higher than that in normal subjects ($P > 0.05$). The ROC curve analysis revealed that CDC2 had a high value in the diagnosis of osteosarcoma (AUC = 0.785, 95% CI = 0.729-0.834) (Fig. 1 and Table IIIA and B).

Clinical features of 47 patients with osteosarcoma. Of the 47 patients, 23 cases were osteoblastic; 13 were osteogenic; and 11, were fibroblastic osteosarcoma, respectively. In osteosarcoma cells, the expression level of CDC2 had no difference in terms of sex, age and occurrence site ($P > 0.05$). Osteosarcoma was divided into 3 levels: Parosteal (I), periosteal (II) and conventional osteosarcoma (III) according to the fourth edition of World Health Organization (WHO) bone tumor classification. The expression level of CDC2 was increased with the increase of level ($P < 0.05$). KPS was scored according to the evaluation standards of physical condition (20). The results showed that the expression level of *CDC2* was increasingly higher with the decrease of KPS score. The CDC2 expression level was closely associated with tumor diameter ($P < 0.05$). Finally, the expression level of CDC2 was increased with the increase of tumor lymph nodes metastasis (TNM) staging ($P < 0.05$) (Table IV).

Table III. Expression levels of CDC2 in tissues and blood.

A, Tissue

Cancer tissue	Paracancerous tissue	P-value
2.31±0.306	0.91±0.251	0.013

B, Blood

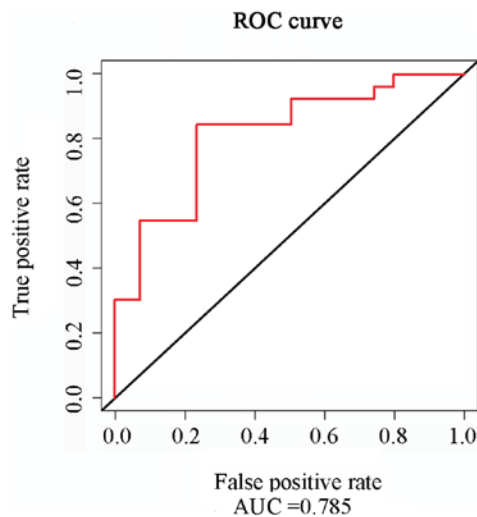| Patient	Normal subject	P-value
1.58±0.149	0.67±0.136	0.024

CDC2, cell division cycle gene 2.

Figure 1. ROC curve of diagnosis of osteosarcoma via CDC2. AUC =0.785 with a high diagnostic value. ROC, receiver operating characteristic; CDC2, cell division cycle gene 2.

Association of TNM staging and CDC2 level with survival rate of patients and its effect on prognosis. The median expression level of CDC2 in 47 patients with osteosarcoma was 2.49. Thus, the patients were divided into the high-expression CDC2 (>2.49) and low-expression CDC2 (<2.49) groups. The univariate and multivariate Cox regression analysis revealed that the increase of the TNM staging of osteosarcoma and the high expression of CDC2 were both risk factors affecting the prognosis of osteosarcoma patients (P<0.05) (Table V).

Discussion

Since the 21st century, it has been found (21) that the abnormal regulation of cell cycle is one of the most important causes of tumor. Researchers have concluded that cancer is a progressive disease that is caused by the destruction of the cell cycle regulation mechanism, and many genes are found to be involved in the cell cycle regulation, providing many important targets for the treatment of cancer (22).

CDC (23) is one of the genes that has been identified, the most important being *CDC2*, and the CDC2 kinase (24) encoded by it controls the beginning of the cell cycle and the transition from the G2 to the M phase. The cell cycle checkpoint regulates various cell regulators, thus completing the mitosis of cells. Previous findings have shown that the disruption of the function of the cell cycle checkpoint may lead to malignant differentiation of cells and produce tumors (25).

In the present study, the expression of CDC2 in cancer, paracancerous tissues and serum from patients and normal controls were detected via RT-PCR. The results showed that, there was a significant difference in the expression level of CDC2 between cancer and paracancerous tissues (P<0.05), as well as the serum in patients and the normal control group (P<0.05). It was also found that a high CDC2 expression may interfere with normal cell growth and differentiation and cause malignant cell proliferation, and the detection of CDC2 expression in serum may predict the occurrence of osteosarcoma. Leijen *et al* (21) found that the function of the tumor cell checkpoint is incomplete and can trigger an automatic interlocking feedback loop, which leads to further malignant cell growth. The expression level of CDC2 was closely associated with tumor diameter, WHO grading and KPS score, indicating that the expression level of CDC2 is closely associated with the occurrence and development of osteosarcoma. Chae *et al* (26) reported that the expression of CDC2 is significantly different between benign and malignant breast lesions, and the increase of CDC2 levels is associated with tumor invasiveness. The results in the present study also showed that the expression level of CDC2 was associated with the TNM staging of osteosarcoma (P<0.05), suggesting that a high expression of CDC2 in osteosarcoma may promote the development of osteosarcoma, and the detection of CDC2 expression in serum may predict the development of osteosarcoma. Yang *et al* (27) found that CDC2 is associated with squamous cell carcinoma of the larynx. The multivariate Cox regression analysis of the prognosis of osteosarcoma patients revealed that the expression level of CDC2 was a risk factor affecting the prognosis of patients with osteosarcoma (P<0.05), making it possible to predict the prognosis of osteosarcoma by detecting the CDC2 expression level in serum. Jansen *et al* (18) found that *CDC2* plays a crucial role in G2 cell cycle progression and cell proliferation, and CDC2 may be considered as a prognostic marker for metastatic breast cancer.

Since no relevant reports are currently available to confirm the clinical significance of CDC2 expression in osteosarcoma, and the sample size was small in this study with a lack of representativeness, a larger number of samples are needed to confirm the findings. In this study, whether patients received chemotherapy and radiotherapy was not recorded; thus, further verification is needed in future research.

Collectively, CDC2 is highly expressed in osteosarcoma tumor cells. A high expression of CDC2 may be involved in the process of tumor development and progression, which leads to disordered mitosis and malignant proliferation of cells. The detection of CDC2 expression in serum may predict the occurrence, development and prognosis of osteosarcoma.

Table IV. Clinical characteristics of 447 patients with osteosarcoma.

Item	No.	CDC2 expression level	P-value
Sex			
Male	31	2.38±0.317	0.685
Female	16	2.24±0.305	
Age (years)			
<12	25	2.31±0.313	0.314
≥12	22	1.91±0.206	
Histological subtypes			
Osteoblastic osteosarcoma	23	2.11±0.331	0.412
Osteogenic osteosarcoma	13	1.98±0.285	
Fibroblastic osteosarcoma	112.06±0.446		
KPS score			
<70	30	3.01±0.363	0.032
≥70	17	1.81±0.106	
Tumor location			
Upper limb bone	92.43±0.278	0.647	
Lower limb bone	38	2.31±0.306	
Tumor size (cm)			
<10	36	2.84±0.267	0.042
≥10	111.94±0.348		
WHO classification			
I	15	1.57±0.124	
II	24	2.79±0.217	0.039
III	81.97±0.135		
TNM staging			
I/II	35	1.65±0.152	0.035
III/IV	12	2.87±0.225	
Pathological fracture			
Yes	112.48±0.165		0.752
No	36	2.74±0.274	

CDC2, cell division cycle gene 2, TNM, tumor lymph nodes metastasis; WHO, World Health Organization.

Table V. Univariate and multivariate analysis.

Variables	Univariate HR (95%CI)	Multivariate P-value	HR (95%CI)	P-value
CDC2 (low vs. high)	1.647 (1.122-2.896)	0.009	1.969 (0.9505-4.0765)	0.012
Age (<12 vs. ≥12 years)	1.014 (0.999-1.029)	0.062		
Sex (male vs. female)	0.819			
(0.277-2.424)	0.788			
Diameter of tumor (<10 vs. ≥10 cm)	0.812			
(0.357-1.847)	0.682			
Tumor site	2.2611			
(Upper vs. lower limb bone)				
(0.9821-147.3)	0.052			
TNM staging	3.064			
(1.282-7.323)	0.028	1.268 (0.918-2.471)	0.041	

CDC2, cell division cycle gene 2, TNM, tumor lymph nodes metastasis.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

GH and BC wrote the manuscript and assisted with PCR. WX and KL designed the primer sequences and analyzed specimens of patients. HZ and HY contributed significantly to statistical analysis. All authors read and approved the final manuscript.

Ethics approval and consent to participate

The study was approved by the Ethics Committee of The Third Affiliated Hospital of Sun Yat-sen University (Guangzhou, China), and the patients or their families signed informed consent.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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