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Original article

The expression of *TSSC3* and its prognostic value in patients with osteosarcoma

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ABSTRACT

Osteosarcoma is one of the most common primary bone tumors in children and adolescents, typically presenting with poor prognosis. Recent studies have found that *TSSC3* had a potential capability in suppressing the tumor development in osteosarcoma. Our purpose was to explore the role of *TSSC3* in the clinical outcome of osteosarcoma patients. Firstly, we detected the expression of *TSSC3* at mRNA level by quantitative real-time polymerase chain reaction (qRT-PCR). The result demonstrated that *TSSC3* expression was lower in osteosarcoma patients than in healthy controls ($P < 0.05$). Then, the relationship between *TSSC3* and clinicopathological characteristics was analyzed by chi-square test which manifested that WHO grade, metastasis, and recurrence were vital influential factors on the expression of *TSSC3* ($P < 0.05$). We also estimated the association between *TSSC3* and overall survival of osteosarcoma patients by Kaplan–Meier analysis as well as assessed the prognostic value of *TSSC3* and clinicopathological characteristics through cox regression analysis. Patients with high *TSSC3* expression were proved to live longer than those with low *TSSC3* expression (log rank test, $P < 0.05$). *TSSC3* expression ($P = 0.032$, HR = 0.405, 95%CI = 0.177–0.926) and metastasis ($P = 0.010$, HR = 2.849, 95% CI = 1.291–6.287) were considered to be independent prognostic factors in osteosarcoma. Taken together, our findings provided preliminary evidence that the *TSSC3* was a prognostic marker in osteosarcoma and this might be useful for the therapy of osteosarcoma.

1. Introduction

Osteosarcoma, the most common primary bone malignancy, is one of the main causes of cancer-related death in children and adolescents [1,2]. It has a high rate of metastasis and relapse which lead to a low cure rate and a low 5-year survival rate. Recently, as the development of neoadjuvant chemotherapy, the 5-year survival rate has raised to 60–70% [3]. However, the prognosis of patients with recurrence and metastasis is still poor. Molecular target therapy for tumors has been employed in the clinical setting which will facilitate and broaden the underlying application prospects of tumor target therapy in future [4]. Therefore, it is necessary to find a novel and reliable gene marker for predicting and improving the prognosis of osteosarcoma.

The occurrence and development of osteosarcoma have a close correlation with the alterations of chromosomal regions, inactivation of tumor suppressor genes and the deregulation of major signaling pathways [5]. Some imprinted oncogenes were verified to be changed in the tumorigenesis of osteosarcoma [6]. Tumor-suppressing STF cDNA 3 (*TSSC3*) is located within the tumor suppressor region of 11p15 and the first apoptosis-related gene shown to be imprinted [7]. The loss of *TSSC3* has been confirmed to regulate the placental growth [8]. In previous studies, the expression of *TSSC3* had been reported to be abnormal in several cases of malignant diseases, such as Wilms'tumor, hydatidiform mole, and human brain tumors [9–11]. In addition, the abnormal *TSSC3* expression had also been confirmed to be related to the growth inhibition, apoptosis induction and improvements of chemotherapeutic effects as well as stemness decrease in osteosarcoma [12–14]. However, the prognostic value of *TSSC3* expression in osteosarcoma is not well understood.

In this study, we attempted to detect the expression of *TSSC3* at mRNA level and estimate its prognostic value in osteosarcoma. Besides we expected to verify the function of *TSSC3* and provide a new therapeutic strategy for patients with osteosarcoma.

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2. Materials and methods

2.1. Patients and tissue samples

This study was conducted in School of Life Sciences and Technology and permitted by the Ethnic Committee of the hospital. 97 patients diagnosed as osteosarcoma were collected and none of them had received any chemotherapy or radiotherapy prior to surgery. The same number healthy people matched with age and sex were taken as controls. Written informed consents had been signed by each participator in advance.

The tissues from osteosarcoma patients and healthy controls were collected. Then the tissues were quickly frozen in liquid nitrogen and stored at −80°C for RNA extraction. Clinicopathological characteristics including age, sex, WHO grade, KPS score, the status of metastasis and recurrence of all patients are recorded in Table 1. A 5-year follow-up was performed. The overall survival was defined from the day of surgery to the death day. Patients who died from unexpected events or other diseases were excluded from our study.

2.2. RNA extraction and quantitative real-time polymerase chain reaction (qRT-PCR)

Total RNA was extracted from tumors and paired normal bone tissue samples using RNA Trizol (Takara, China). QRT-PCR was performed with a One Step SYBR® PrimeScript® RT-PCR Kit (Takara, China) following the manufacturer's instruction. β-actin was amplified as endogenous controls. The sequences of the primers were as follows: *TSSC3*: forward 5′-ACC GCC TGA GCC TGT TCC C-3'and reverse 5′-CTG GCG GCT GCG AAA GTC C-3′ [13]; β-actin forward 5′-TGA CGT GGA CAT CCG CAA AG-3'and reverse 5′-CTG GAA GGT GGA CAG CGA GG-3′ [15]. The expression of *TSSC3* normalized to β-actin at mRNA level was evaluated by the comparative cycle threshold (CT) method. Each sample was in triplicate.

2.3. Statistical analysis

All computations were carried out using the software of SPSS version 18.0 for Windows (SPSS Inc, IL, USA). The difference of *TSSC3* expression between osteosarcoma tissues and paired normal tissues was evaluated by Students' *t* test. Chi-Square test was used to analyze the relationship between *TSSC3* expression and the clinicopathological characteristics. The association between *TSSC3* expression and overall survival of patients was analyzed via Kaplan–Meier analysis. Cox regression analysis was taken to estimate the prognostic value of *TSSC3* in osteosarcoma. The difference was considered to be statistically significant when the value of *P* was less than 0.05.

Table 1
The relationship between TSSC3 expression and clinicopathological characteristics.

Characteristics	Cases (*n*)	TSSC3 expression		*P* value
		High (*n* = 50)	Low (*n* = 47)	
Age				0.335
<20	55	26	29	
≥20	42	24	18	
Gender				0.896
Male	53	27	26	
Female	44	23	21	
WHO grade				0.010
Low (I,II)	44	29	15	
High (III,IV)	53	21	32	
Metastasis				0.020
Absent	49	31	18	
Present	48	19	29	
Recurrence				0.008
Absent	38	26	12	
Present	59	24	35	
KPS				0.131
<90	46	20	26	
≥90	51	30	21	

Note: KPS, Karnofsky performance score,when the score ≥90 indicates that patients had a good health.

3. Results

3.1. TSSC3 expression was decreased in osteosarcoma tissues

The expression of *TSSC3* at mRNA level was detected using qRT-PCR. In the 97 osteosarcoma tissues, 50 patients exhibited high *TSSC3* expression while 47 with low *TSSC3* expression. The qRT-PCR results indicated that the expression level of *TSSC3* was lower in osteosarcoma tissue (0.236 ± 0.015) than that in paired normal tissues (0.638 ± 0.022, $P < 0.05$, Fig. 1). It demonstrated that *TSSC3* might be a tumor suppressor in osteosarcoma.

3.2. The correlation between TSSC3 expression and clinicopathological characteristics in osteosarcoma patients

In order to explore the role of *TSSC3* in the development of osteosarcoma, we analyzed the relationship between the expression of *TSSC3* and the clinicopathological characteristics. The results were summarized in Table 1. *TSSC3* expression was influenced by WHO grade of the tumor ($P = 0.010$), metastasis ($P = 0.020$) and recurrence ($P = 0.008$). However, there was no correlation between *TSSC3* and other clinicopathological characteristics such as age, gender, and KPS. These might reveal that *TSSC3* participated in the development and progress of osteosarcoma.

3.3. The expression of TSSC3 was associated with the overall survival of patients with osteosarcoma

The follow-up lasted for 60 months and the median follow-up time was 38 months. During the follow-up, 21 of 47 patients with low *TSSC3* expression (44.68%) and 14 of 50 patients with high *TSSC3* expression patients (28%) had died. Kaplan–Meier analysis indicated that patients with high *TSSC3* expression had a longer overall survival time than those with low *TSSC3* expression (log rank test, $P < 0.05$, Fig. 2). Besides, multivariate analysis was carried out to estimate the prognostic value of *TSSC3* and clinicopathological characteristics. The outcome showed that tumor metastasis ($P = 0.010$, HR = 2.849, 95%CI = 1.291–6.287) and *TSSC3* expression ($P = 0.032$, HR = 0.405, 95%CI = 0.177–0.926) were

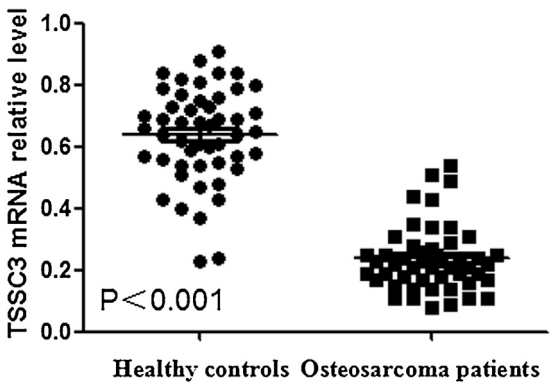

Fig. 1. The expression of *TSSC3* in osteosarcoma patients and healthy controls. It was lower in osteosarcoma tissues than healthy controls ($P < 0.05$).