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Original Article

Decreased expression of long non-coding RNA MEG3 acts as a potential predictor biomarker in progression and poor prognosis of osteosarcoma

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Abstract: Introduction: Long non-coding RNA MEG3 (lncRNA MEG3) has been showed to involve in a variety of cancers. However, the association between lncRNA MEG3 expression level and the prognosis of osteosarcoma is still unclear. Methods: The expression levels of lncRNA MEG3 in osteosarcoma tissues and adjacent non-tumor tissues were detected using quantitative real-time PCR (qRT-PCR). Differences in patient survival were determined using the Kaplan-Meier method and a log-rank test. A Cox proportional hazards regression analysis was used for univariate and multivariate analyses of prognostic values. Results: Our findings showed that expression of lncRNA MEG3 was clearly lower in osteosarcoma tissues compared with adjacent non-tumor tissues. The expression of lncRNA MEG3 was associated with clinical stage and distant metastasis ($P<0.05$). Kaplan-Meier analysis showed that patients with low lncRNA MEG3 expression had a shorter overall survival (log-rank test, $P<0.05$). Furthermore, multivariate analysis revealed that decreased expression of lncRNA MEG3, advanced clinical stage and distant metastasis were all independent predictors to overall survival of osteosarcoma patients. Conclusions: Downregulation of lncRNA MEG3 was associated with poor overall survival of osteosarcoma. LncRNA MEG3 could be a useful biomarker for progression and prognosis of osteosarcoma.

Keywords: lncRNA MEG3, osteosarcoma, progression, prognosis

Introduction

Osteosarcoma is the most common primary malignancy in children and adolescents, accounting for 20 to 35% of all malignant primary bone tumors [1, 2]. Though advances of modern treatments such as surgery, chemotherapy, and the combination of surgery and chemotherapy are improved, long-term survival rate of patients diagnosed with advanced osteosarcoma remains very low [3, 4]. Therefore, it is of great significance to investigate the molecular mechanisms involved in osteosarcoma, and to identify novel and effective targets to improve therapeutic efficacy and clinical outcome for osteosarcoma.

Long non coding RNA (lncRNA) is a type of RNA molecule with length of more than 200 nucleotides and lacks an open reading frame of significant length and the capability of coding protein [5, 6]. A large number of studies showed that lncRNA act as key molecules regulating gene expression, chromatin remodeling, transcription, and post-transcriptional processing [7, 8]. In addition, increasing numbers of reports showed that the disorders of lncRNA are closely related to human diseases, especially cancer [9]. For example, Gupta et al showed that lncRNA HOTAIR was increased in breast tumors and could promote metastasis by the interaction with the Polycomb Repressive Complex 2 [10]. Zhang et al showed that clear cell renal cell carcinoma patients with higher SPRY4-IT1 expression had an advanced clinical stage and poorer prognosis, knockdown of SPRY4-IT1 reduced renal cancer cell proliferation, migration, and invasion [11]. Shi et al reported that GAS5 was downregulated in non-small-cell lung carcinoma and GAS5 overexpression could induce apoptosis and growth arrest, In addition, they found that p53 and E2F1 were key downstream mediators of GAS5 [12]. However, the role of lnRNAs in the development of osteosar-

Table 1. Correlation between lncRNA MEG3 expression and clinicopathological features of osteosarcoma

Clinicopathological features	Group	Total	MEG3 expression		*P* value
			Low	High	
Gender	Male	36	19	17	0.614
	Female	28	13	15	
Age (years)	<25	40	18	22	0.302
	≥25	24	14	10	
Tumor size (cm)	<8 cm	37	22	15	0.076
	≥8 cm	27	10	17	
Anatomic location	Tibia/femur	44	24	20	0.281
	Elsewhere	20	8	12	
Clinical stage	I/II	31	10	21	0.006
	III	33	22	11	
Distant metastasis	Absence	47	19	28	0.011
	Presence	17	13	4	

coma remains ambiguous and further studies is needed.

In this study, we investigated the expression of lncRNA MEG3 in osteosarcoma, and analyzed the association of its expression with clinicopathological features and clinical prognosis.

Materials and methods

Patient samples

This study was approved by the Research Ethics Committee of Xinxiang Central Hospital. Written informed consent was obtained from all of the patients. In total, we recruited 64 patients with osteosarcomas from Department of Orthopedics, Xinxiang Central Hospital between May 2005 and April 2007. None of the patients enrolled in this study had received radiotherapy or chemotherapy before surgery. The clinical stage of these osteosarcoma patients was classified according to the sixth edition of the TNM classification of the Union for International Cancer Control (UICC). The clinicopathological information of the patients is summarized in **Table 1**.

Quantitative real-time PCR

The total RNA isolated from frozen samples using TRIzol reagent (Invitrogen) based on the constructor's instructions. For quantitative real-time PCR (qRT-PCR), RNA was reverse-transcribed to cDNA by using a reverse transcription kit (Takara). qRT-PCR was performed with Power SYBR Green (Takara). Results were normalized to the expression of GAPDH. The PCR primers for MEG3 or GAPDH were as folows: MEG3 sense, 5'-CTGCCCATCTACACCTCACG-3' and reverse, 5'-CTCTCCGCCGTCTGCGCTAGGGGCT-3'; GAPDH sense, 5'-GTCAACGGATTTGGTCTGTATT-3' and reverse, 5'-AGTCTTCTGGGTGGCAGTGAT-3'. qRT-PCR and data collection were performed on ABI 7500. The relative expression of MEG3 was calculaed and normalized using the $2^{-\Delta\Delta Ct}$ method relative to GA PDH.

Statistical analysis

We used SPSS 18.0 software for statistical analysis. Differences between groups were evaluated using Student's t-test and χ^2 test. Survival analysis was done by using the log-rank test and Kaplan-Meier method. Moreover, a Cox proportional hazards model was performed to evaluate prognostic values of clinicopathological features. $P<0.05$ was statistically significant.

Results

Expression of lncRNA MEG3 is down-regulated in osteosarcoma

To test the effect of lncRNA MEG3 on tumor progression, the expression levels of lncRNA MEG3 in tumor tissues and paired non-tumor tissues from 64 osteosarcoma patients were measured by qRT-PCR. As shown in **Figure 1A**, lncRNA MEG3 expression was significantly decreased in osteosarcoma tissues compared to adjacent non-tumor tissues ($P<0.05$). These data suggested that lncRNA MEG3 could act as a tumor suppressor to prevent progression of osteosarcoma.

Association between lncRNA MEG3 expression and clinicopathological features

According to the median expression level of lncRNA MEG3, we categorized the patients into

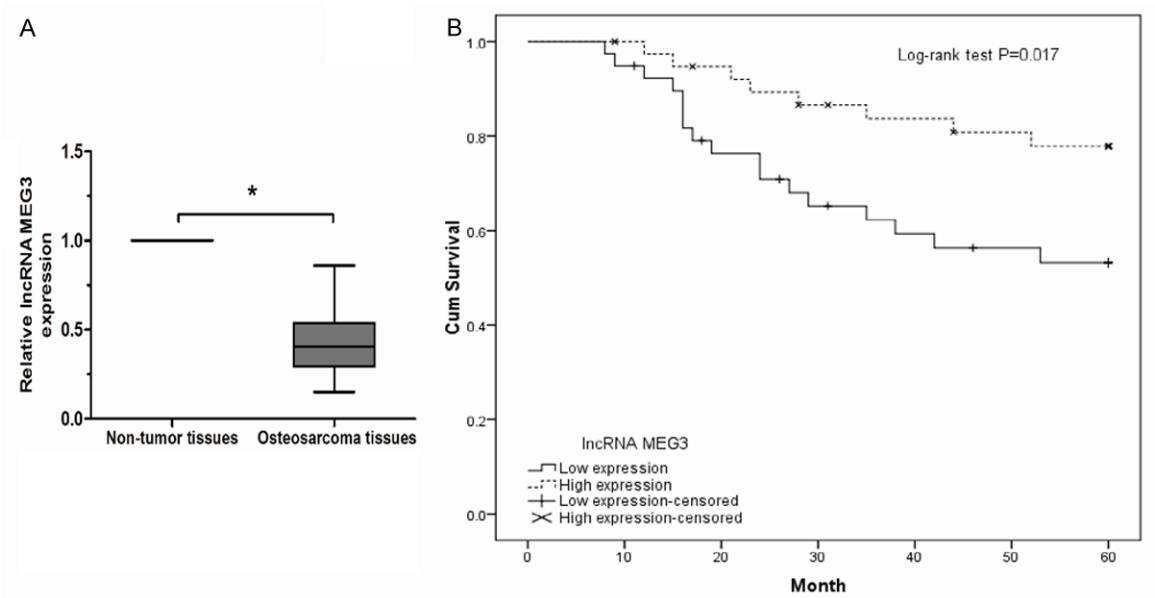

Figure 1. Relative lncRNA MEG3 expression in osteosarcoma tissues and its clinical significance. A. Relative lncRNA MEG3 expression level in osteosarcoma tissues and adjacent non-tumor tissues; the expression of level of lncRNA MEG3 was detected in osteosarcoma patients by qRT-PCR. B. Kaplan-Meier overall survival curves according to lncRNA MEG3 expression level. *P<0.05.

Table 2. Univariate analysis of clinicopathological features for overall survival of osteosarcoma patients

Clinicopathological features	Univariate analysis		
	Hazard ratio	95% CI	*P*
Gender	1.316	0.518-3.182	0.373
Male vs. Female			
Age (years)	0.975	0.593-2.861	0.406
≥25 vs. <25			
Tumor size	2.137	0.759-6.068	0.098
≥8 cm vs. <8 cm			
Anatomic location	1.134	0.665-3.672	0.184
Elsewhere vs. Tibia/femur			
Clinical stage	2.945	1.815-6.963	0.004
III vs. I/II			
Distant metastasis	3.716	2.183-8.476	0.002
Presence vs. Absence			
MEG3	2.735	1.573-7.718	<0.001
Low vs. High			

low and high expression groups. The correlation between clinicopathological features and lncRNA MEG3 expression in high and low expression groups were summarized in **Table 1**. Low lncRNA MEG3 expression was observed to be closely correlated with advanced clinical stage and distant metastasis (P<0.05). However, there was no association between lncRNA MEG3 expression and other clinical features, such as gender, age, tumor size, and anatomic location (P>0.05).

Association between lncRNA MEG3 expression and prognosis in osteosarcoma patients

As shown in **Figure 1B**, Kaplan-Meier analysis showed that the overall survival rate of the osteosarcoma patients with low lncRNA MEG3 expression was significantly lower than that of patients with high lncRNA MEG3 expression (log-rank test, P<0.05). Next, univariate and multivariate analyses were utilized to evaluate whether the lncRNA MEG3 expression level and various clinicopathological features were independent prognostic features of osteosarcoma patient outcomes. Univariate analysis showed that lncRNA MEG3 expression, clinical stage, and distant metastasis were significantly associated with overall survival of osteosarcoma patients (**Table 2**, P<0.05). Furthermore, multivariate Cox regression analyses revealed that lncRNA MEG3 expression, clinical stage, and distant metastasis were

Table 3. Multivariate analysis of clinicopathological features for overall survival of osteosarcoma patients

Clinicopathological features	Multivariate analysis		
	Hazard ratio	95% CI	*P*
Clinical stage	2.713	1.592-6.311	0.015
III vs. I/II			
Distant metastasis	3.358	1.885-7.392	0.011
Presence vs. Absence			
MEG3	2.415	1.318-6.806	0.006
Low vs. High			

independent prognostic factors for osteosarcoma patients (**Table 3**, P<0.05).

Discussion

The role of lnRNAs in development of osteosarcoma remains ambiguous and discovery of new specific therapeutic targets may provide effective management of disease. Dysreulation of different lncRNAs have been previously suggested in osteosarcoma, For example, Sun et al showed that increased expression of lncRNA HULC was associated with poor prognosis and promoted cell metastasis in osteosarcoma [13]. Zhang et al revealed that downregulation of lncRNA TUG1 inhibited osteosarcoma cell proliferation and promoted apoptosis [14]. Dong et al suggested that lncRNA MALAT1 could promote the proliferation and metastasis of osteosarcoma cells by activating the PI3K/Akt pathway [15]. In the present study, we evaluate the expression of lncRNA MEG3 in term of osteosarcoma.

Maternally expressed gene 3 (MEG3), which encoded a non-coding RNA, is an imprinted gene belonging to the DLK1-MEG3 locus located on chromosome 14q32.3 in humans with maternal expression [16]. MEG3 RNA was expressed in many normal tissues, while it was lost in an expanding list of primary human tumors [17]. For example, Lu et al showed that lncRNA MEG3 was decreased in non-small cell lung cancer and associated with advanced clinical features. Furthermore, patients with lower expression of lncRNA MEG3 had a significantly poor prognosis [18]. Yin et al reported that decreased expression of lncRNA MEG3 could promote cell proliferation and predicted a poor prognosis in colorectal cancer patients [19]. Sun et al found that downregulated lncRNA MEG3 was associated with poor prognosis and promoted cell proliferation in gastric cancer [20].

In the present study, our results showed that expression of lncRNA MEG3 was significantly decreased in osteosarcoma, suggesting that lncRNA MEG3 may function as tumor suppressor. Furthermore, we found that lncRNA MEG3 expression was associated with clinical stage and distant metastasis, indicating that lncRNA MEG3 might be involved in the carcinogenesis and metastasis of osteosarcoma. More important, we found patient with low expression of lncRNA MEG3 was significantly associated with a shorter overall survival time, suggesting that low lncRNA MEG3 level is a biomarker of poor prognosis for osteosarcoma patients. However, the precise molecular mechanisms behind the altered expression of lncRNA MEG3 in osteosarcoma and its function are not very clear. Therefore, additional studies are needed to more clearly and comprehensively articulate the molecular mechanisms of both the cause and the effects of altered expression of lncRNA MEG3 in the progression of osteosarcoma.

In conclusion, our result showed that lncRNA MEG3 may function in suppression of tumor whose downregulation of lncRNA MEG3 can be associated with progression and metastasis of osteosarcoma and would applied as a therapeutic agent.

Disclosure of conflict of interest

None.

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