European Review for Medical and Pharmacological Sciences 2018; 22: 4115-4120

High expression of long non-coding RNA LOC730101 correlates with distant metastasis and exhibits a poor prognosis in patients with osteosarcoma

S.-W. LI[1], K.-Z. HU[2], S.-C. CHEN[2], S.-L. LIU[3], Y.-H. WANG[4]

[1]Department of Clinical Laboratory, Yinan Country People's Hospital, Linyi, Shandong, China
[2]Department of Ophthalmology, Shanxian Central Hospital, Heze, Shandong, China
[3]Department of Clinical Laboratory, Changle County People's Hospital, Weifang, Shandong, China
[4]Department of nursing, Anqiu City People's Hospital, Anqiu, Shandong, China

Shou-wei Li and Ke-zheng Hu contributed equally to this work

Abstract. – OBJECTIVE: Expression of long non-coding RNA LOC730101 (LOC730101) has been closely linked to the carcinogenesis of osteosarcoma and has been shown to function as a tumor promoter. However, the clinical significance of LOC730101 remains unknown. Our present study aimed to investigate the feasibility of LOC730101 as a novel prognostic biomarker for osteosarcoma.

PATIENTS AND METHODS: Quantitative Real-time RT-PCR (qRT-PCR) was performed to examine LOC730101 expression in osteosarcoma tissues and adjacent non-cancerous tissues. The correlation between LOC730101 expression and clinicopathological features and the overall survival rate was determined. Cox regression analyses were performed to explore whether LOC730101 was an independent predictor of survival for osteosarcoma patients.

RESULTS: We found that LOC730101 was significantly upregulated in human osteosarcoma tissues compared with corresponding adjacent normal tissues ($p < 0.01$). Elevated LOC730101 expression was correlated with advanced clinical stage ($p = 0.001$) and distant metastasis ($p = 0.005$). Furthermore, survival assay revealed that osteosarcoma patients in the high LOC730101 expression group had significantly shorter 5-year overall survival time than those in the low LOC730101 expression group ($p = 0.0002$). In addition, multivariate analysis showed that LOC730101 maintained an independent prognostic influence on overall survival ($p = 0.006$).

CONCLUSIONS: Our present study, for the first time, revealed that LOC730101 may serve as a poor prognostic indicator in patients with osteosarcoma.

Key Words:
Long noncoding RNA, LOC730101, Prognosis, Osteosarcoma.

Introduction

Osteosarcoma is the most common bone malignancy in the pediatric population, and it could occur in any bone of an individual[1]. It is characterized by a highly malignant and metastatic potential (especially to lung)[2]. The survival rates of the non-metastatic osteosarcoma patients have increased to 65% with the development treatment approaches of osteosarcoma such as wide tumor excision, chemotherapy, and curative resection[3,4]. However, the 5-year overall survival of patients with local relapse or distant metastasis is only 20-40%[5]. Besides, early diagnosis is associated with the prognosis of osteosarcoma patients[6]. Thus, there is an unmet need to explore new diagnostic and prognostic markers to improve the clinical management of osteosarcoma patients.

The research of long non-coding RNAs (lncRNAs) has recently gained prominence in the field of disease progression[7]. LncRNAs are more than 200 nt in length with little protein-coding potential[8]. Long ago, lncRNAs initially thought to represent spurious transcriptional noise. However, in the past few years, lncRNAs have emerged as novel mechanisms in mediating tumorigenesis and progression of cancer[9,10]. In

Corresponding Author: Yu-hua Wang, MD; e-mail: yy616111@126.com

addition, more and more evidence shows that lncRNAs participate in cancer cell biological processes including cell growth, cell invasion, and cell metastasis[11,12]. For instance, Wang et al[13] reported that lncRNA SNHG12 was highly expressed in non-small cell lung cancer and its knockdown suppressed cell growth via modulating miR-138 in vitro and in vivo. Zhang et al[14] showed that lncRNA NEAT1 expression was significantly up-regulated and associated with prognosis of breast cancer patients. Functionally, suppression of lncRNA NEAT1 could suppress proliferation and metastasis of breast cancer cells via modulating β-catenin and EMT. In osteosarcoma, lnc-SNHG1 was reported to play an oncogenic role in osteosarcoma because its forced expression could promote osteosarcoma cell proliferation, migration, and EMT process[15]. The important role of lncRNAs in cancer progression revealed lncRNAs as potential biomarkers for cancer diagnosis and prognosis prediction in many solid tumors[16,17].

LncRNA LOC730101 (LOC730101), a newly identified lncRNA associated with tumor development, has been reported to be dysregulated in several tumors. However, up to date, only two studies[18,19] reported the specific biological function of LOC730101 by performing in vitro experiments. In osteosarcoma, Cheng et al[18] firstly identified LOC730101 as one of up-regulated lncRNA in osteosarcoma patients. Moreover, the carcinogenic role of LOC730101 was confirmed by in vitro and in vivo assay. However, whether it is associated with clinical prognosis has not been investigated.

Patients and Methods

Tissue Specimens

Osteosarcoma tissues and matched normal tissues were obtained from 244 patients with osteosarcoma that was confirmed by histopathological analysis at the Yinan Country People's Hospital and the Anqiu City People's Hospital, between 2011 and 2012. No patient received chemotherapy or radiotherapy prior to surgery. Histological diagnoses were made according to the 2007 WHO classification. The time between the diagnostic biopsy and surgery date to death or last follow-up was defined as overall survival duration. The clinicopathological features of patients were shown in Table I. Written-informed consent was obtained from each patient, and the study was approved by the Institute Research Medical Ethics Committee of the Yinan Country People's Hospital and the Anqiu City People's Hospital.

RNA Extraction and Real-Time Quantitative PCR

Total RNA from human tissues was extracted using the TRIzol reagent (Applied Biosystems, Foster City, CA, USA) according to the manufacturer's instructions. The RNA quality and concentration were determined using the Nanodrop 2000 spectrophotometer. The reverse transcription of lncRNA into cDNA was made with the use of PrimeScript RT Reagent Kit (Invitrogen, Carlsbad, CA, USA). LOC730101 expression was determined by performing qRT-PCR with SYBR Premix Ex Taq (TaKaRa, Otsu, Shiga, Japan) and 7500 Real-time PCR system (Applied Biosystems, Foster City, CA, USA). The relative expression of LOC730101

Table I. Correlation between LOC730101 expression with clinicopathologic features of osteosarcoma.

			LOC730101 level		
Parameters	**Category**	**No.**	**High**	**Low**	***p*-value**
Age (year)	<25	111	51	60	NS
	≥25	134	69	64	
Gender	Male	148	70	78	NS
	Female	96	50	46	
Tumor size	>8 cm	101	58	43	NS
	≤8 cm	143	62	81	
Anatomical location	Tibia/femur	133	63	70	NS
	Elsewhere	111	57	54	
Clinical stage	IIA	140	56	84	0.001
	IIB/III	104	64	40	
Distant metastasis	Absent	146	61	85	0.005
	Present	98	59	39	

was normalized using glyceraldehyde phosphate dehydrogenase (GAPDH). All experiments were performed using the $2^{-\Delta\Delta Ct}$ method. The primer sequences were as follows: LOC730101: Forward, 5'- TTGCCATGGGTGGTAGCTTT-3'; Reverse: 5'-GTGAGACGAACGGTAGGGTG-3'. GAPDH: Forward: 5'-AGAAGGCTGGGGCTCATTTG-3'; Reverse: 5'-AGGGGCCATCCACAGTCTTC-3'. Each sample was analyzed in triplicate.

Statistical Analysis

Statistical Product and Service Solutions 17.0 (SPSS Inc., Chicago, IL, USA) was used for the data processing. LOC730101 expression in 244 pairs of primary osteosarcoma tissues and corresponding adjacent tissues was compared by Wilcoxon signed-rank test. The x^2-test was used to assess LOC730101 expression with respect to clinicopathological factors. The overall survival curves were determined using the Kaplan-Meier method, and the log-rank test was used to compare the survival curves. Univariate and multivariate analyses were conducted to explore the independent risk factors for osteosarcoma using the Cox proportional hazard model. The value of $p < 0.05$ was considered statistically significant.

Results

Increased LOC730101 Expression in Osteosarcoma Tissues

To investigate the role of LOC730101 in osteosarcoma progression, we detected the expression levels of LOC730101 in osteosarcoma tissues and matched normal tissues via qRT-PCR. It was observed that the expression of LOC730101 was significantly up-regulated in osteosarcoma tissues as compared with adjacent normal bone tissues ($p < 0.01$) (Figure 1). Above results may indicate that LOC730101 may serve as tumor promoter gene in osteosarcoma.

LOC730101 Upregulation Associates With Advanced Clinicopathological Features of Human Osteosarcoma

Then, we further explored the association between LOC730101 levels and clinicopathological features of human osteosarcoma. Accordingly, the median value of LOC730101 was used as a cutoff value to divide all 244 patients into two groups: the high expression group (n = 120) and the low expression group (n = 124). Pearson x^2-test was performed and the results showed that LOC730101 upregulation was significantly correlated with advanced clinical stage ($p = 0.001$) and positive distant metastasis ($p = 0.005$). However, there were no significant associations between LOC730101 expression and age, gender, tumor size, and anatomical location ($p > 0.05$).

Upregulation of LOC730101 Associates With Poor Prognosis of Human Osteosarcoma

To explore the prognostic value of LOC730101 for patients with osteosarcoma, overall survival was assessed by Kaplan-Meier survival analysis. We found that patients with decreased LOC730101

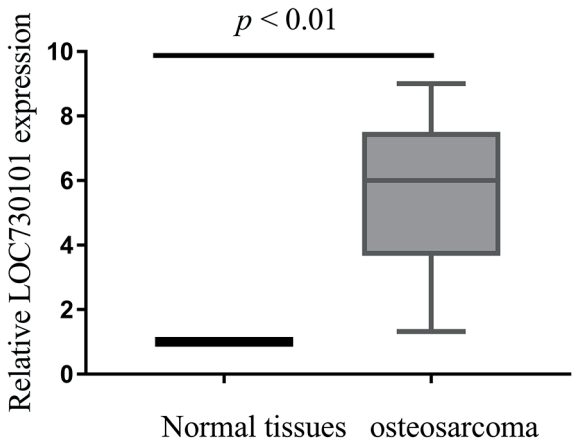

Figure 1. Expression of LOC730101 in osteosarcoma tissues and matched normal tissues was detected by RT-PCR. LOC730101 expression was significantly higher in osteosarcoma tissues than in the matched normal samples ($p < 0.01$).

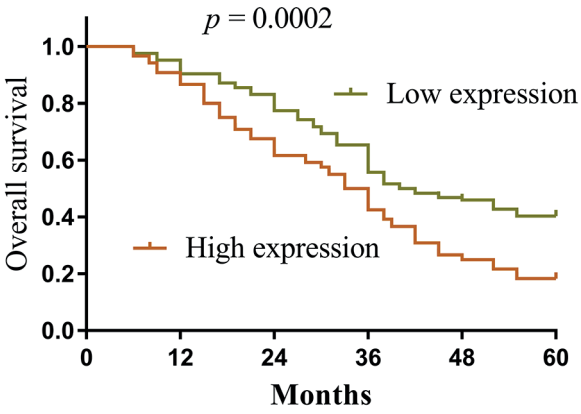

Figure 2. Kaplan-Meier curves for overall survival in patients with osteosarcoma divided according to LOC730101 expression. Patients with high LOC730101 expression had poorer overall survival compared with low LOC730101 group ($p = 0.0002$).

Table II. Prognostic factors in Cox proportional hazards model.

	Univariate analysis			Multivariate analysis		
Variable	**RR**	**95% CI**	***p***	**RR**	**95% CI**	***p***
Age <25 vs. ≥25	1.213	0.767-1.655	0.543	-	-	-
Gender Male vs. Female	1.539	0.832-1.943	0.238	-	-	-
Tumor size >8 vs. ≤8	1.775	0.856-2.323	0.123	-	-	-
Anatomical location Tibia/femur vs. Elsewhere	1.932	1.232-4.667	0.177	-	-	-
Clinical stage IIA vs. IIB/III	4.458	1.674-8.832	0.001	3.325	1.235-5.534	0.008
Distant metastasis Absent vs. Present	3.459	1.776-5.439	0.006	3.013	1.237-4.325	0.026
LOC730101 expression Low vs. High	4.562	1.948-7.347	0.003	3.789	1.323-6.436	0.006

expression had better overall survival than those with LOC730101 up-regulation ($p = 0.0002$) (Figure 2). Moreover, Cox regression analyses were conducted to evaluate the prognostic factors in 244 osteosarcoma patients. As shown in Table II, univariate analysis revealed that clinical stage ($p = 0.001$), distant metastasis ($p = 0.006$), and LOC730101 expression ($p = 0.003$) were prognostic indicators. In addition, variables with a value of $p < 0.05$ were selected for multivariate analysis. The results showed that clinical stage (HR, 3.325; 95% CI, 1.235-5.534; $p = 0.008$), distant metastasis (HR, 3.013; 95% CI, 1.237-4.325; $p = 0.026$), and LOC730101 expression (HR, 3.789; 95% CI, 1.323-6.436; $p = 0.006$) predicted poor prognosis independently.

Discussion

The pathogenic factors of osteosarcoma are very complex and have not been fully revealed yet. Thus, it is very hard to develop the effective therapeutic method. Clinical practice and various studies confirmed that early diagnosis could improve the outcome and quality of life of osteosarcoma patients[20]. Besides, accurate prediction of osteosarcoma patients who have a potential higher risk of recurrence, metastasis, and resistance to chemotherapy is very important for the option of therapeutic method and the timing of the treatment[21,22]. Right now, several clinicopathological features were used for the prognostic prediction of osteosarcoma patients. However, the specificity of this system remains relatively low[23]. Thus, it is urgent to identify effective markers to improve prognosis prediction of osteosarcoma and to develop better diagnosis system of osteosarcoma patients.

Accumulating evidence[24,25] has revealed that dysregulation of lncRNAs exhibits close correlation with several kinds of human tumors, including osteosarcoma. With the development of high-throughput sequencing, the siftings of dysregulated lncRNAs in tumor became easy, making lncRNAs a research hotspot in exploring potential diagnostic and prognostic biomarkers[26]. Recently, growing studies confirmed lncRNAs expression was significantly associated with overall survival of osteosarcoma patients. For instance, Tian et al[27] reported that lncRNA MEG3 was lowly expressed in osteosarcoma and its low expression was associated with poor prognosis. Cai et al[28] found that lncRNA HNF1A-AS1 levels in blood could be used for distinguishing osteosarcoma from healthy individuals. Prognostic assay revealed that lncRNA HNF1A-AS1 was closely related to the prognosis of osteosarcoma. Recently, a novel lncRNA, LOC730101, was reported to be over-expressed in lung cancer tissues and cell lines and to serve as a tumor promoter by promoting lung cancer cell growth via modulating Wnt canonical pathway[19]. Then, Cheng et al[18] also observed that LOC730101 was a tumor-promotive gene in osteosarcoma. However, the expression pattern of LOC730101 should also be confirmed in more clinical samples of

osteosarcoma. Besides, the prognostic value of LOC730101 has not been investigated.

In this study, our results of RT-PCR also showed that the expression of LOC730101 was increased in osteosarcoma tissues as compared to adjacent non-tumor tissues. Furthermore, potential associations of LOC730101 expression with clinicopathological factors were examined and the results revealed that LOC730101 upregulation was significantly correlated with advanced clinical stage and positive distant metastasis. Subsequently, the results from the cohort 2 survival analyses suggested that high LOC730101 expression was remarkably related to overall survival of osteosarcoma patients, suggesting LOC730101 as a potential biomarker for predicting the prognosis of osteosarcoma patients. Interestingly, LOC730101 expression levels were further analyzed by Cox regression analysis. Our results confirmed that high expression of LOC730101 may be an independent prognosis factor for overall survival. However, the precise mechanism by which LOC90784 influence the clinical outcome of LOC730101 requires further investigation in the future. At the same time, further evaluations of larger samples are needed to confirm our findings.

Conclusions

Our data demonstrated that LOC730101 was increased in patients with osteosarcoma. Besides, increased LOC730101 levels were correlated with advanced clinical stage and poor prognosis of osteosarcoma. To our best knowledge, our study is the first to identify LOC730101 as a useful marker for clinical outcome of osteosarcoma patients.

Conflict of Interest

The Authors declare that they have no conflict of interest.

References

1) Mirabello L, Troisi RJ, Savage SA. Osteosarcoma incidence and survival rates from 1973 to 2004: data from the Surveillance, Epidemiology, and End Results Program. Cancer 2009; 115: 1531-1543.
2) Osborne TS, Khanna C. A review of the association between osteosarcoma metastasis and protein translation. J Comp Pathol 2012; 146: 132-142.
3) Geller DS, Gorlick R. Osteosarcoma: a review of diagnosis, management, and treatment strategies. Clin Adv Hematol Oncol 2010; 8: 705-718.
4) Kobys VL, Konovalenko VF, RepinÐ NV, Golovko TS, Gulak LO, Tarasova TO, Zaharycheva EV, Matyushok OF. Treatment of large osteosarcoma in children: new approach. Exp Oncol 2013; 35: 105-108.
5) Kunz P, Fellenberg J, Moskovszky L, Sápi Z, Krenacs T, Machado I, Poeschl J, Lehner B, Szendrői M, Ruef P, Bohlmann M, Bosch AL, Ewerbeck V, Kinscherf R, Fritzsching B. Improved survival in osteosarcoma patients with atypical low vascularization. Ann Surg Oncol 2015; 22: 489-496.
6) Li C, Cong Y, Liu X, Zhou X, Zhou G, Lu M, Shi X, Wu S. The progress of molecular diagnostics of osteosarcoma. Front Biosci (Landmark Ed) 2016; 21: 20-30.
7) Marchese FP, Huarte M. Long non-coding RNAs and chromatin modifiers: their place in the epigenetic code. Epigenetics 2014; 9: 21-26.
8) Krishnan J, Mishra RK. Emerging trends of long non-coding RNAs in gene activation. FEBS J 2014; 281: 34-45.
9) Shi X, Sun M, Liu H, Yao Y, Song Y. Long non-coding RNAs: a new frontier in the study of human diseases. Cancer Lett 2013; 339: 159-166.
10) Li CH, Chen Y. Targeting long non-coding RNAs in cancers: progress and prospects. Int J Biochem Cell Biol 2013; 45: 1895-1910.
11) Johnsson P, Lipovich L, Grandér D, Morris KV. Evolutionary conservation of long non-coding RNAs; sequence, structure, function. Biochim Biophys Acta 2014; 1840: 1063-1071.
12) Ponting CP, Oliver PL, Reik W. Evolution and functions of long noncoding RNAs. Cell 2009; 136: 629-641.
13) Wang X, Qi G, Zhang J, Wu J, Zhou N, Li L, Ma J. Knockdown of long noncoding RNA small nucleolar RNA Host Gene 12 inhibits cell growth and induces apoptosis by upregulating mir-138 in nonsmall cell lung cancer. DNA Cell Biol 2017; 36: 892-900.
14) Zhang M, Wu WB, Wang ZW, Wang XH. lncRNA NEAT1 is closely related with progression of breast cancer via promoting proliferation and EMT. Eur Rev Med Pharmacol Sci 2017; 21: 1020-1026.
15) Jiang Z, Jiang C, Fang J. Up-regulated lnc-SNHG1 contributes to osteosarcoma progression through sequestration of miR-577 and activation of WNT2B/Wnt/β-catenin pathway. Biochem Biophys Res Commun 2018; 495: 238-245.
16) Shao Y, Ye M, Jiang X, Sun W, Ding X, Liu Z, Ye G, Zhang X, Xiao B, Guo J. Gastric juice long noncoding RNA used as a tumor marker for screening gastric cancer. Cancer 2014; 120: 3320-3328.
17) Wen JJ, Ma YD, Yang GS, Wang GM. Analysis of circulating long non-coding RNA UCA1 as potential biomarkers for diagnosis and prognosis of osteosarcoma. Eur Rev Med Pharmacol Sci 2017; 21: 498-503.

18) Cheng G, Li B, Sun X, Cao Z, Zhang G, Zhao Z, Zhao Y, Yu Q, Liu W. LncRNA LOC730101 promotes osteosarcoma cell survival under energy stress. Biochem Biophys Res Commun 2018; 496: 1-6.

19) Liu L, Zhang Y, Cao W. Highly expressed lncRNA LOC730101 promotes lung cancer cell growth through Wnt canonical pathway. Biochem Biophys Res Commun 2017; 493: 992-997.

20) Moore DD, Luu HH. Osteosarcoma. Cancer Treat Res 2014; 162: 65-92.

21) Seker MM, Seker A, Aksoy S, Ozdemir N, Uncu D, Zengin N. Clinicopathologic features and prognosis of osteosarcoma in Turkish adults. Asian Pac J Cancer Prev 2014; 15: 3537-3540.

22) Isakoff MS, Bielack SS, Meltzer P, Gorlick R. Osteosarcoma: Current Treatment and a Collaborative Pathway to Success. J Clin Oncol 2015; 33: 3029-3035.

23) Nouri H, Ben Maitigue M, Abid L, Nouri N, Abdelkader A, Bouaziz M, Mestiri M. Surface osteosarcoma: Clinical features and therapeutic implications. J Bone Oncol 2015; 4: 115-123.

24) Wang D, Gao ZM, Han LG, Xu F, Liu K, Shen Y. Long noncoding RNA CASC2 inhibits metastasis and epithelial to mesenchymal transition of lung adenocarcinoma via suppressing SOX4. Eur Rev Med Pharmacol Sci 2017; 21: 4584-4590.

25) Sun J, Wang X, Fu C, Wang X, Zou J, Hua H, Bi Z. Long noncoding RNA FGFR3-AS1 promotes osteosarcoma growth through regulating its natural antisense transcript FGFR3. Mol Biol Rep 2016; 43: 427-436.

26) Ilott NE, Ponting CP. Predicting long non-coding RNAs using RNA sequencing. Methods 2013; 63: 50-59.

27) Tian ZZ, Guo XJ, Zhao YM, Fang Y. Decreased expression of long non-coding RNA MEG3 acts as a potential predictor biomarker in progression and poor prognosis of osteosarcoma. Int J Clin Exp Pathol 2015; 8: 15138-15142.

28) Cai L, Lv J, Zhang Y, Li J, Wang Y, Yang H. The lncRNA HNF1A-AS1 is a negative prognostic factor and promotes tumorigenesis in osteosarcoma. J Cell Mol Med 2017; 21: 2654-2662.