

RESEARCH ARTICLE

Long intergenic non-coding RNA 511 correlates with improved prognosis, and hinders osteosarcoma progression both in vitro and in vivo

Suchi Qiao^{1,2} | Ke Qi³ | Chang Liu⁴ | Changli Xu³ | Jun Ma² | Xinmin Xu^{1,2} | Cheng Li³ | Zhiwei Wang³ 

¹The Outpatient Department, 905 Hospital of Navy, Second Military Medical University, Shanghai, China

²Department of Orthopedics, Changzheng Hospital, Second Military Medical University, Shanghai, China

³Department of Orthopedics, Changhai Hospital, Second Military Medical University, Shanghai, China

⁴Department of Orthopedics, 900 Hospital of the Joint Logistics Team, Fuzhou, China

Correspondence

Zhiwei Wang and Cheng Li, Department of Orthopedics, Changhai Hospital, Second Military Medical University, 168 Changhai Road, Shanghai 200433, China.
Emails: bei48192442@163.com (ZW); chennacaox9z@163.com (CL)

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Abstract

Background: This study aimed to investigate the correlation of long intergenic non-coding RNA 511 (LINC00511) with clinicopathological characteristics and overall survival (OS) in osteosarcoma patients and to explore its function in osteosarcoma in vitro and in vivo.

Methods: Tumor tissues and adjacent tissues from 45 osteosarcoma patients were acquired, and LINC00511 expression was detected. In vitro, LINC00511 overexpression-treated (OE-LINC00511) and nonsense overexpression-treated (OE-control) MG-63 and Saos-2 cells were cultured, followed by the assessment of cell proliferation, apoptosis, migration, and invasion. In vivo, tumor weight and volume were measured in OE-LINC00511 and OE-control xenografted mice.

Results: LINC00511 expression was decreased in tumor tissues compared with adjacent tissues ($P < .001$), and its high expression correlated with increased tumor cell necrosis rate to neoadjuvant chemotherapy ($P = .025$) and prolonged OS ($P = .010$). In vitro, LINC00511 expression was decreased in osteosarcoma cell lines (including MG-63, U-2OS, Saos-2, and HOS) compared with osteoblast cell line (All $P < .001$). Cell proliferation was decreased at 48 hours (Both $P < .01$) and 72 hours (Both $P < .001$) (in MG-63 and Saos-2 cells); cell apoptosis was increased ($P < .05$) (in Saos-2 cells); cell migration and invasion were decreased (All $P < .01$) (in MG-63 cells and Saos-2 cells) in OE-LINC00511 compared with OE-control. In vivo, tumor volume was reduced at week 4 ($P < .001$), week 5 ($P < .001$), week 6 ($P < .001$) in OE-LINC00511 compared with OE-control. Tumor weight was declined in OE-LINC00511 than OE-control ($P < .001$).

Conclusions: LINC00511 acts as a potential biomarker and therapeutic option for osteosarcoma.

Qiao and Qi contributed equally to this work.

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KEYWORDS

Cell motility, cell survival, long intergenic non-coding RNA 511, osteosarcoma, tumor progression

1 | INTRODUCTION

Osteosarcoma, a kind of tumor originating from mesenchyma, has been reported to be the most common primary sarcoma of bone in young individuals and in elderly adults, which typically presents with a bimodal age distribution.^{1,2} Although the introduction of adjuvant or neoadjuvant chemotherapy accompanying with surgical treatment has greatly increased the 5-year survival rate from less than 20% to approximately 70% in patients osteosarcoma, their prognosis is still far from satisfaction with high rate of recurrence or metastasis.³⁻⁵ Therefore, it is necessary to identify potential therapeutic target to improve prognosis in osteosarcoma patients.

Long non-coding RNAs (lncRNAs) are a group of non-protein coding RNAs with the length exceeding 200 nucleotides, which display multiple biological functions (including chromatin modification, transcriptional regulation, and post-transcriptional regulation),^{6,7} and previous research reveals that lncRNA presents with potential as prognostic biomarker for cancer.⁸ Among the reported lncRNAs, long intergenic non-coding RNA 511 (LINC00511) is proposed to be an oncogene in several cancer cells (such as bladder, breast, and lung cancer cells), and its high expression is correlated with worse prognosis in patients with different cancers (such as liver cancer and ovarian cancer).⁹⁻¹³ These previous studies suggest that LINC00511 is highly expressed in tumor tissues and acts as an oncogene in several cancers, whereas its function in osteosarcoma still remains unclear. In our preliminary study, we found that LINC00511 expression was decreased in osteosarcoma tissues compared with adjacent tissues, which was inconsistent with previous studies, and further confirmation was necessary. Therefore, we performed this study to investigate the correlation of LINC00511 with clinicopathological characteristics and overall survival (OS) in osteosarcoma patients and to explore its function in osteosarcoma both *in vitro* and *in vivo*.

2 | MATERIALS AND METHODS

2.1 | Patients

Forty-five patients with osteosarcoma who underwent surgery in our hospital from January 2010 to January 2015 were consecutively recruited in this study. The inclusion criteria were as follows: (a) pathologically diagnosed as osteosarcoma confirmed by preoperative puncture or open biopsy; (b) single lesion without distant metastases at initial diagnosis; (c) lesion located at the extremities; (d) there was no other underlying disease that affected the course of osteosarcoma treatment; (e) completed neoadjuvant chemotherapy and adjuvant chemotherapy in our hospital or designated cooperative

hospital; (f) underwent limb salvage or amputation operation in our hospital; and (g) patients and their families agreed to participate in this study. The exclusion criteria were as follows: (a) secondary osteosarcoma; (b) the primary lesion was an organ other than bone; (c) lesion located in the axial bone; (d) presenting with multiple lesions, distant metastases, or Enneking stage III at initial diagnosis; (e) had a history of malignancies; and (f) failure to complete all standard treatment. This study was approved by the Ethics Committee of Changhai Hospital, Second Military Medical University, and all patients or their guardians provided the written informed consents.

2.2 | Specimen collection

Osteosarcoma tissue of enrolled patients was obtained through CT-guided puncture biopsy or open biopsy, and the adjacent normal tissue was acquired by surgical resection. After sampling, the osteosarcoma tissue and the adjacent normal tissue were immediately placed in pre-marked specimen bottles and preserved in liquid nitrogen and then transferred to the specimen bank of our department (stored at -80°C). The LINC00511 expressions in the osteosarcoma tissue and the adjacent normal tissue were determined by the Reverse Transcription-Quantitative Polymerase Chain Reaction (RT-qPCR).

2.3 | Treatment

After the initial diagnosis was established, neoadjuvant chemotherapy using IOR-OS/N-5 regimen was administered to all patients, which was conducted as follows: Day 1: high-dose methotrexate (HD-MTX) $10\text{--}12\text{ g/m}^2$ intravenous drip within 5 hours, followed by intravenous injection of sodium folinate at 6 hours, 12 hours, 18 hours, 24 hours, 30 hours, 36 hours, 42 hours, 48 hours, 54 hours, 60 hours, 66 hours, and 72 hours (the complete of HD-MTX intravenous drip was defined as 0 hour), meanwhile, MTX concentration in plasma was monitored at 0 hour, 12 hours, 24 hours, 48 hours, and 72 hours; Day 8: adriamycin (ADM) $20\text{ mg/m}^2/\text{d} \times 3$ days intravenous drip; Day 11: cisplatin (DDP) 120 mg/m^2 intravenous drip; Day 21: ifosfamide (IFO) $2\text{ g/m}^2/\text{d} \times 5$ days intravenous drip, using Mesna to prevent hemorrhagic cystitis. Each cycle repeated every 3 weeks. A total of two cycles of IOR-OS/N-5 regimen were given to patients before surgery. When the neoadjuvant chemotherapy was completed, patients received amputation or limb-salvage surgery, which was decided with the consideration of patients' Enneking stage, sensitivity to neoadjuvant chemotherapy, tumor features, and willingness. After surgery, the IOR-OS/N-5 regimen was given to

the patients who were sensitive to the neoadjuvant chemotherapy, while the intensive IOR-OS/N-5 regimen (IFO dosage was increased from $2 \text{ g/m}^2/\text{d} \times 5 \text{ days}$ to $3 \text{ g/m}^2/\text{d} \times 5 \text{ days}$, with etoposide (VP16) $120 \text{ mg/m}^2/\text{d} \times 3 \text{ days}$ added) was administered to the patients who were insensitive to the neoadjuvant chemotherapy.

2.4 | Assessment of sensitivity to neoadjuvant chemotherapy and tumor cell necrosis rate (TCNR)

The patients' sensitivity to neoadjuvant chemotherapy was assessed through the tumor volume change before and after neoadjuvant chemotherapy. At the initial diagnosis and after the completion of neoadjuvant chemotherapy, each patient underwent MRI enhanced scan to determine the long axis, coronal axis and sagittal axis of the tumor, and the tumor volume was estimated according to the ellipsoidal model formula: $V = \text{PI}/6 \times \text{long axis} \times \text{coronal axis} \times \text{sagittal axis}$. And the tumor volume change was calculated by the ratio of the volume at initial diagnosis and the volume post neoadjuvant chemotherapy. The ratio less than 95% was identified as volume reduced; the ratio between 95% and 105% was identified as volume unchanged; the ratio more than 105% was identified as volume increased. Based on the tumor volume change, patients with the tumor volume reduced or unchanged were defined as sensitive to the neoadjuvant chemotherapy, correspondingly, patients with the tumor volume increased were identified as insensitive to neoadjuvant chemotherapy. The TCNR was evaluated by the HE staining. The section with the largest tumor area and the most complex signal on preoperative MRI was selected as staining section and fixed with 10% formalin for 24 hours, followed by the decalcification of 5% nitric acid, then HE staining was performed. Using the Huvos grading system, TCNR was classified as follows: Huvos I: there was almost no tumor necrosis; Huvos II: TCNR >50%; Huvos III: TCNR >90%; Huvos IV: no living tumor cells was found in all sections.

2.5 | Clinical data collection

Patients' clinical data were documented during diagnosis and treatment, which included age, gender, tumor location, Enneking stage, surgery type, sensitivity to neoadjuvant chemotherapy, and TCNR. After discharge from the hospital, patients were followed up every 3 months for the first 2 years, every 4 months in the third year, then every 6 months for the fourth and fifth year. And the survival data were collected for the assessment of OS, which was measured from the date of initial treatment to the date of death; patients not known to have died at last follow-up were censored on the date they were last known to be alive.

2.6 | Cell culture

Human osteoblast cell line hFOB1.19 and human osteosarcoma cell lines including MG-63, U-2OS, Saos-2, and HOS were purchased from

OBiO Technology (Shanghai) Corp., Ltd. The hFOB1.19 cells were cultured in 90% DMEM/F12 Medium supplemented with 10% fetal bovine serum (FBS) (HyClone). The MG-63 and HOS cells were cultured in 90% MEM α Medium (Gibco) supplemented with 10% FBS (HyClone). The U-2OS and Saos-2 cells were cultured in 90% McCoy's 5A (Modified) Medium (Gibco) supplemented with 10% FBS (HyClone). 293T cells were also purchased from OBiO Technology (Shanghai) Corp., Ltd and were cultured in 90% DMEM Medium (HyClone) supplemented with 10% FBS (HyClone). All cells were incubated at 37°C in a humid atmosphere with 95% air and 5% CO₂. The LINC00511 expression in the cells was detected by the RT-qPCR.

2.7 | Lentivirus packaging and infection

The recombinant plasmid was constructed using synthetic target gene DNA (or nonsense DNA) fragment and the expression vector pLenti-EF1a-EGFP-F2A-Puro-CMV-MCS (OBiO Technology (Shanghai) Corp., Ltd). After sequencing verification, the recombinant plasmid was extracted and purified. Subsequently, the recombinant plasmid, the lentivirus envelope plasmid, and lentivirus packaging plasmid were co-transfected into 293T cells using HilyMax (Dojindo) to generate overexpression lentivirus. Then, the supernatant containing lentivirus was collected and ultra-centrifuged. Next, the MG-63 cells and Saos-2 cells were infected with the overexpression lentivirus, and after 24 hours incubation, the puromycin (Sigma) was added to the culture medium for 7 days to screen stably infected cells. The cells infected with LINC00511 overexpression (OE) lentivirus were identified as OE-LINC00511 cells. Also, the cells infected with nonsense overexpression lentivirus were marked as OE-control cells. Besides, the MG-63 cells and Saos-2 cells without lentivirus infection were used as blank-control. The LINC00511 expression in OE-LINC00511, OE-control, and blank-control cells was determined by the RT-qPCR; the proliferation of the cells was measured by CCK-8 assay at 0 hour, 24 hours, 48 hours, and 72 hours; the cell apoptosis was assessed by the Annexin V-APC/7-AAD assay at 48 hours; migration ability and invasive ability were determined by wound healing assay and transwell assay.

2.8 | Tumor xenograft construction

Male BALB/c-nude mice with age 6 ~ 7 weeks were obtained from the Nanjing Biomedical Research Institute, and all animal experiments were performed in accordance with a protocol approved by the Institutional Animal Care and Use Committees (IACUC) of the Nanjing Biomedical Research Institute. All mice were raised in the barrier environment, with the temperature 20-26°C, the relative humidity 40%-70% and 14:10 hours alternating lighting, and free to drinking and eating. Mice were fed adaptively for 2 ~ 7 days before experiment. The 1×10^7 transfected Saos-2 cells (OE-LINC00511 and OE-control) and Saos-2 blank-control cells were subcutaneously injected into the armpit of the anterior right limb of nude mice,

and then the mice were accordingly divided into three groups (OE-LINC00511, OE-control, and blank-control) with five mice in each group. The long diameter and short diameter of subcutaneous tumor in nude mice were measured weekly using a vernier caliper, and the tumor volume was calculated according to the formula: tumor volume = $1/2 \times$ tumor long diameter \times square of tumor short diameter. At the 6th week of the experiment, nude mice were sacrificed, subcutaneous tumor grafts were isolated and weighed.

2.9 | RT-qPCR

Total RNA extraction was performed using TRIzol™ Reagent (Invitrogen). Reverse transcription was conducted using PrimeScript™ RT Master Mix (Takara), and qPCR was performed using SYBR® Premix Dimer Eraser™ (Takara) following the manufacturer's guidance. β -actin was set as internal reference. The relative gene expression was calculated by the $2^{-\Delta\Delta Ct}$ method. Primers: LINC00511, forward primer (5' → 3'): GACTACTGTTACCTCGCTTGGGA, reverse primer (5' → 3'): CTGGCATGTGAGCACCTGTA; β -actin, forward primer (5' → 3'): CACTCTTCCAGCCTTCCTTCTCT, reverse primer (5' → 3'): ACTCGTCATACTCTGCTTGCT.

2.10 | CCK-8 assay

The proliferation was determined using CCK-8 kits (OBIo Technology (Shanghai) Corp., Ltd, China) following the manufacturer's instructions. Briefly, a solution containing 10 μ L of CCK-8 solution and 100 μ L of medium was added to each well at the ending time (0 hour, 24 hours, 48 hours or 72 hours) and the cells were subsequently cultured at 37°C for 2 hours. The optical density (OD) value of each well at 450 nm was read using a microplate reader (TECAN).

2.11 | Annexin V-APC/7-AAD assay

After being harvested, cells were stained with Annexin V-allophycocyanin (APC) and 7-Amininactinomycin D (7-AAD) for 15 minutes in the dark using an Annexin V-APC/7-AAD apoptosis kit (MultiSciences). The apoptosis rate was determined using a flow cytometer (Thermo Scientific) and Flowjo 7.0 software (Flowjo, LLC).

2.12 | Wound healing assay

OE-LINC00511 cells, OE-control cells, and blank-control cells were cultured in medium without FBS (HyClone) for 24 hours. A 10- μ L tip was used to make a wound. After being washed twice with phosphate-buffered saline, cells were cultured in medium supplemented with 2% FBS. The average gaps of the cells were monitored at 0 hour and 24 hours, and the photographs were captured (100 \times) using an inverted microscope (Olympus). The migration rate was analyzed and calculated by image-Pro Plus (Media Cybernetics).

2.13 | Transwell assay

The Transwell chambers (Costar) were hydrated using culture medium. Cells were seeded onto the upper surface of the Transwell chambers. After 24 hours, the cells on the upper surface of the chambers were removed and the cells on the lower surface of the chambers were fixed with 4% formaldehyde (Sigma) and stained with crystal violet (Sigma). Photographs were captured at 24 hours using a microscope (Olympus).

2.14 | Statistical analysis

Data were displayed as mean and standard deviation (SD), median and interquartile range (IQR), and count (percentage). Using the median value of tumor LINC00511 expression as cutoff value, patients were divided into the LINC00511 high expression group and the LINC00511 low expression group. Comparison of LINC00511 expression between tumor and adjacent tissue was determined by the Wilcoxon signed-rank test. Comparison of characteristics between two groups was determined by Student's test or chi-square test; comparison among different groups was determined by the Dunnett's multiple comparisons test. Overall Survival was displayed using Kaplan-Meier curve and determined by log-rank test. Factors affecting OS were analyzed by univariate and multivariate Cox's proportional hazard regression model. SPSS 20.0 (IBM) and GraphPad prism 6.00 (GraphPad) were used for data analysis and graph plotting. *P* value < .05 was considered as significant.

3 | RESULTS

3.1 | Clinical characteristics of osteosarcoma patients

The mean age of osteosarcoma patients was 23.0 ± 14.7 years. For clinicopathological characteristics, there were 3 (6.3%) patients with Enneking stage IIA, and 42 (93.3%) patients with Enneking stage IIB. For response to neoadjuvant chemotherapy, there were 20 (44.4%) patients insensitive to neoadjuvant chemotherapy and 25 (55.6%) patients sensitive to neoadjuvant chemotherapy, meanwhile there were 22 (48.9%) patients with Huvos grade I ~ II and 23 (51.1%) patients with Huvos grade III ~ IV. For surgery type, 12 (26.7%) patients underwent amputation and 33 (73.3%) patients underwent limb-salvage. The detailed characteristics of osteosarcoma patients were listed in Table 1.

3.2 | LINC00511 expression in osteosarcoma patients

In order to detect LINC00511 expression in tumor tissues and adjacent tissues, RT-qPCR was performed, which disclosed that it was decreased in tumor tissue compared with adjacent tissue (*P* < .001) (Figure 1).

TABLE 1 Clinical characteristics

Characteristics	Total osteosarcoma patients (N = 45)	LINC00511		t/χ^2	P value
		Low expression patients (n = 23)	High expression patients (n = 22)		
Age (y), M \pm SD	23.0 \pm 14.7	24.74 \pm 16.15	21.27 \pm 13.27	-0.785	.437
Gender, No. (%)				2.905	.088
Male	27 (60.0)	11 (47.8)	16 (72.7)		
Female	18 (40.0)	12 (52.2)	6 (27.3)		
Tumor location, No. (%)				2.197	.568
Distal femur	26 (57.8)	15 (68.2)	11 (50.0)		
Proximal tibia	10 (22.2)	4 (17.4)	6 (27.3)		
Distal tibia	4 (8.9)	1 (4.3)	3 (13.6)		
Proximal humerus	5 (11.1)	3 (13.1)	2 (9.1)		
Enneking stage, No. (%)				0.002	.968
IIA	3 (6.7)	1 (4.3)	2 (9.1)		
IIB	42 (93.3)	22 (95.7)	20 (90.9)		
Surgery type, No. (%)				0.008	.928
Amputation	12 (26.7)	6 (26.1)	6 (27.3)		
Limb-salvage	33 (73.3)	17 (73.9)	16 (69.7)		
Sensitivity to neoadjuvant chemotherapy, No. (%)				1.138	.286
Insensitive	20 (44.4)	12 (52.2)	8 (36.4)		
Sensitive	25 (55.6)	11 (47.8)	14 (63.6)		
TCNR, No. (%)				5.020	.025
Huvos I ~ II	22 (48.9)	15 (65.2)	7 (31.8)		
Huvos III ~ IV	23 (51.1)	8 (34.8)	15 (68.2)		

Note: Comparison was determined by the Student's t test or chi-square test.

Abbreviations: M \pm SD, mean \pm standard deviation; TCNR, tumor cell necrosis rate.

3.3 | Correlation of LINC00511 with clinical characteristics in osteosarcoma patients

According to the median value of LINC00511 expression in tumor tissues, osteosarcoma patients were further divided into LINC00511 high expression patients (n = 22) and LINC00511 low expression patients (n = 23). TCNR was increased in LINC00511 high expression patients compared with LINC00511 low expression patients ($P = .025$), while no difference was observed in age ($P = .437$), gender ($P = .088$), tumor location ($P = .568$), Enneking stage ($P = .968$), surgery type ($P = .928$), or sensitivity to neoadjuvant chemotherapy ($P = .286$) between the two groups (Table 1).

3.4 | Correlation of LINC00511 expression with OS in osteosarcoma patients

To investigate the correlation of LINC00511 expression with OS in osteosarcoma patients, Kaplan-Meier curve was performed, which illustrated that LINC00511 high expression was correlated with prolonged OS in osteosarcoma patients ($P = .010$) (Figure 2).

3.5 | Factors affecting OS in osteosarcoma patients

Univariate Cox's regression revealed that LINC00511 high expression ($P = .009$, hazard ratio [HR] = 0.252), limb-salvage ($P = .025$, HR = 0.293), sensitive to neoadjuvant chemotherapy ($P = .001$, HR = 0.120), and increased level of TCNR ($P = .001$, HR = 0.080) were factors that ameliorate OS in patients with osteosarcoma. Furthermore, multivariate Cox's regression disclosed that LINC00511 high expression ($P = .002$, HR = 0.038) and limb-salvage ($P = .046$, HR = 0.196) were independent factors that prolong OS in patients with osteosarcoma (Table 2).

3.6 | LINC00511 expression in osteosarcoma cell lines and osteoblast cell line

In order to further explore the underlying function of LINC00511 in osteosarcoma, several in vitro experiments were carried out. The relative LINC00511 expression was decreased in osteosarcoma cell lines including Saos-2 ($P < .001$), HOS ($P < .001$), MG-63 ($P < .001$), and U-2OS ($P < .001$) compared with control cell line hFOB1.19 (Figure 3). And we selected MG-63 and Saos-2 cells to perform the following study.

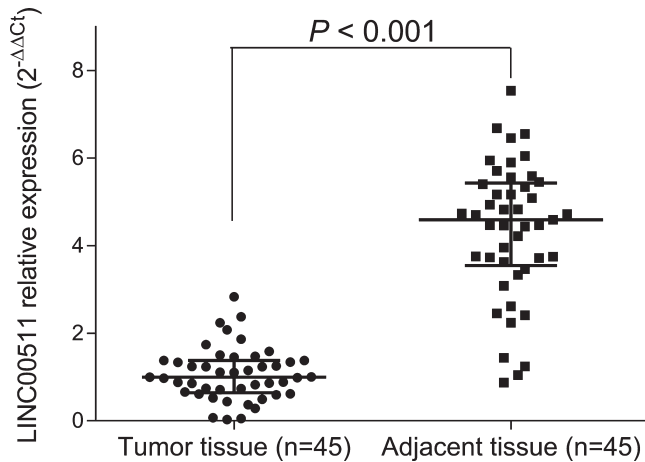


FIGURE 1 LINC00511 expression decreased in tumor tissues compared with adjacent tissues in osteosarcoma patients. Comparison of LINC00511 expression between tumor tissues and adjacent tissues was conducted by the Wilcoxon signed-rank test. P value $< .05$ was considered significant. LINC00511: long intergenic non-coding RNA 511

3.7 | Effect of LINC00511 on cell proliferation and apoptosis

In MG-63 cells, LINC00511 expression was increased in OE-LINC00511 group compared with OE-control group ($P < .001$), indicating successful transfection (Figure 4A). Cell proliferation was decreased in OE-LINC00511 group at 48 hours ($P < .001$) and 72 hours ($P < .001$) compared with OE-control group (Figure 4B). While no difference was found in cell apoptosis between OE-LINC00511 group and OE-control group ($P > .05$) (Figure 4C,D). In Saos-2 cells, LINC00511 expression

was increased in OE-LINC00511 group compared with OE-control group ($P < .001$) suggesting successful transfection (Figure 4E). Cell proliferation was decreased in OE-LINC00511 group at 48 hours ($P < .01$) and 72 hours ($P < .001$) compared with OE-control group (Figure 4F). Meanwhile, cell apoptosis was increased in OE-LINC00511 group ($P < .05$) compared with OE-control group (Figure 4G,H). These data indicated that LINC00511 inhibited cell proliferation while increase apoptosis in osteosarcoma cells.

3.8 | Effect of LINC00511 on cell migration and invasion

Cell migration rate was reduced in OE-LINC00511 group compared with OE-control group in MG-63 cells ($P < .01$) (Figure 5A,B) and Saos-2 cells ($P < .001$) (Figure 5C,D). Meanwhile, invasive cell count was also decreased in OE-LINC00511 group compared to OE-control group both in MG-63 cells ($P < .001$) (Figure 6A,B) and Saos-2 cells ($P < .001$) (Figure 6C,D). These data presented that LINC00511 inhibited cell migration and cell invasion in osteosarcoma cells.

3.9 | Effect of LINC00511 on tumor volume and tumor weight in osteosarcoma xenograft mice

To further explore the function of LINC00511 on tumor volume and tumor weight in osteosarcoma, in vivo experiments were performed. Tumor volume was reduced in OE-LINC00511 group at week 4 ($P < .001$), week 5 ($P < .001$), and week 6 ($P < .001$) compared with OE-control group (Figure 7A,B). As for tumor weight, it was reduced in OE-LINC00511

TABLE 2 Univariate and multivariate Cox's proportional hazard regression model analyses of factors affecting OS

Items	Univariate Cox's regression		Multivariate Cox's regression	
	P value	HR (95% CI)	P value	HR (95% CI)
LINC00511 high expression ^a	.009	0.252 (0.089-0.712)	.002	0.038 (0.005-0.293)
Age (≥ 19 y) ^b	.227	1.833 (0.686-4.893)	.318	2.090 (0.492-8.871)
Gender (male)	.153	2.132 (0.755-6.024)	.863	1.146 (0.243-5.391)
Tumor location				
Distal femur	Reference	-	Reference	-
Proximal tibia	.295	0.503 (0.139-1.819)	.068	0.185 (0.030-1.131)
Distal tibia	.531	1.639 (0.350-7.681)	.085	31.792 (0.619-1633.004)
Proximal humerus	.405	1.971 (0.399-9.733)	.479	0.457 (0.052-4.004)
Enneking stage (IIB vs. IIA)	.592	0.566 (0.071-4.535)	.298	7.717 (0.165-361.515)
Surgery type (Limb-salvage)	.025	0.293 (0.100-0.859)	.046	0.196 (0.039-0.974)
Sensitivity to neoadjuvant chemotherapy (Sensitive)	.001	0.120 (0.034-0.415)	.112	0.072 (0.003-1.842)
TCNR (Huvos III/IV vs. Huvos I/II)	.001	0.080 (0.018-0.349)	.496	0.361 (0.019-6.814)

Abbreviations: CI, confidence interval; HR, hazard ratio; OS, overall survival; TCNR: tumor cell necrosis rate.

^apatients were classified as LINC00511 high expression group and LINC00511 low expression group according to the median value of tumor LINC00511 expression.

^bpatients were classified as two groups according to the median age.

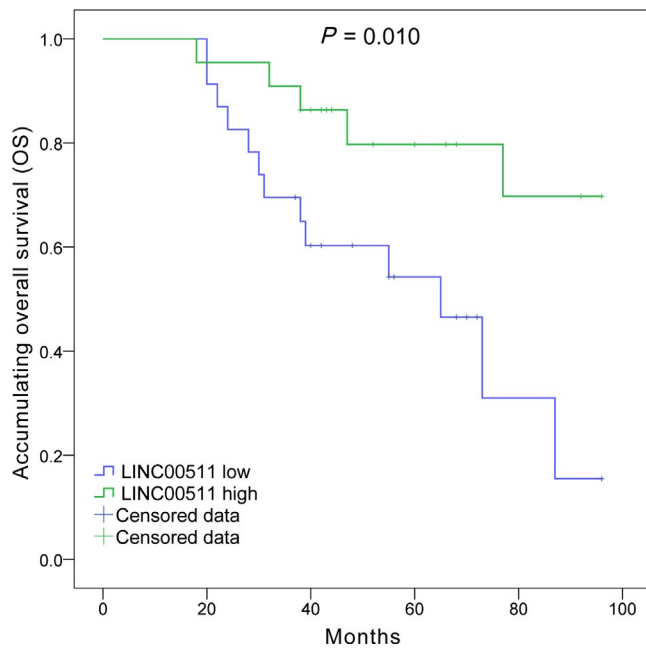


FIGURE 2 LINC00511 high expression correlated with better OS in patients with osteosarcoma. Kaplan-Meier curve was conducted to display OS. Comparison of OS between LINC00511 high expression group and LINC00511 low expression group was conducted by log-rank test. P value $<.05$ was considered significant. LINC00511: long intergenic non-coding RNA 511; OS, overall survival

group compared with OE-control group as well ($P < .001$) (Figure 7C). These data exhibited that LINC00511 suppress tumor growth in vivo.

4 | DISCUSSION

In this study, we discovered that: (a) LINC00511 expression was decreased in osteosarcoma tissues; meanwhile, it was correlated with increased TCNR and better OS. Moreover, LINC00511 high expression was an independent factor that prolonged OS in osteosarcoma patients; (b) LINC00511 decreased proliferation, migration, and invasion, while increased apoptosis of osteosarcoma cells. (c) LINC00511 decreased tumor growth in xenografted mice.

LINC00511 has been discovered to be overexpressed in several tumor tissues, including pancreatic ductal adenocarcinoma (PDAC) tissues, glioma tumor tissues, and lung cancer tissues compared with adjacent normal tissues.^{9,11,14-16} In addition, there are a few studies performed to investigate the correlation of LINC00511 with clinicopathological characteristics in cancer patients. For instance, LINC00511 is positively correlated with lymphatic metastasis in pancreatic ductal adenocarcinoma patients.¹⁵ Another study discovered that LINC00511 is positively associated with tumor size, lymph node metastasis and distant metastasis in triple-negative breast cancer patients.¹⁷ These studies indicate that LINC00511 is overexpressed in tumor tissues and serves as a promoter in different cancer patients; however, the role of LINC00511 in osteosarcoma is yet to be elucidated. In our preliminary study, we found that LINC00511 expression was reduced in osteosarcoma tissues

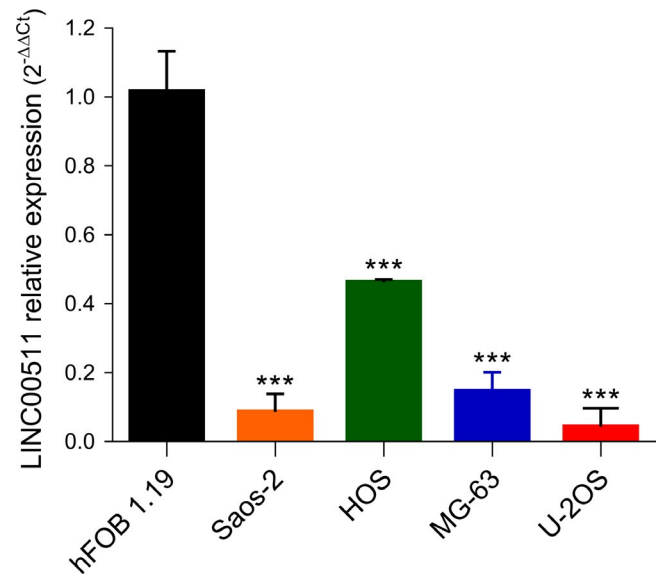
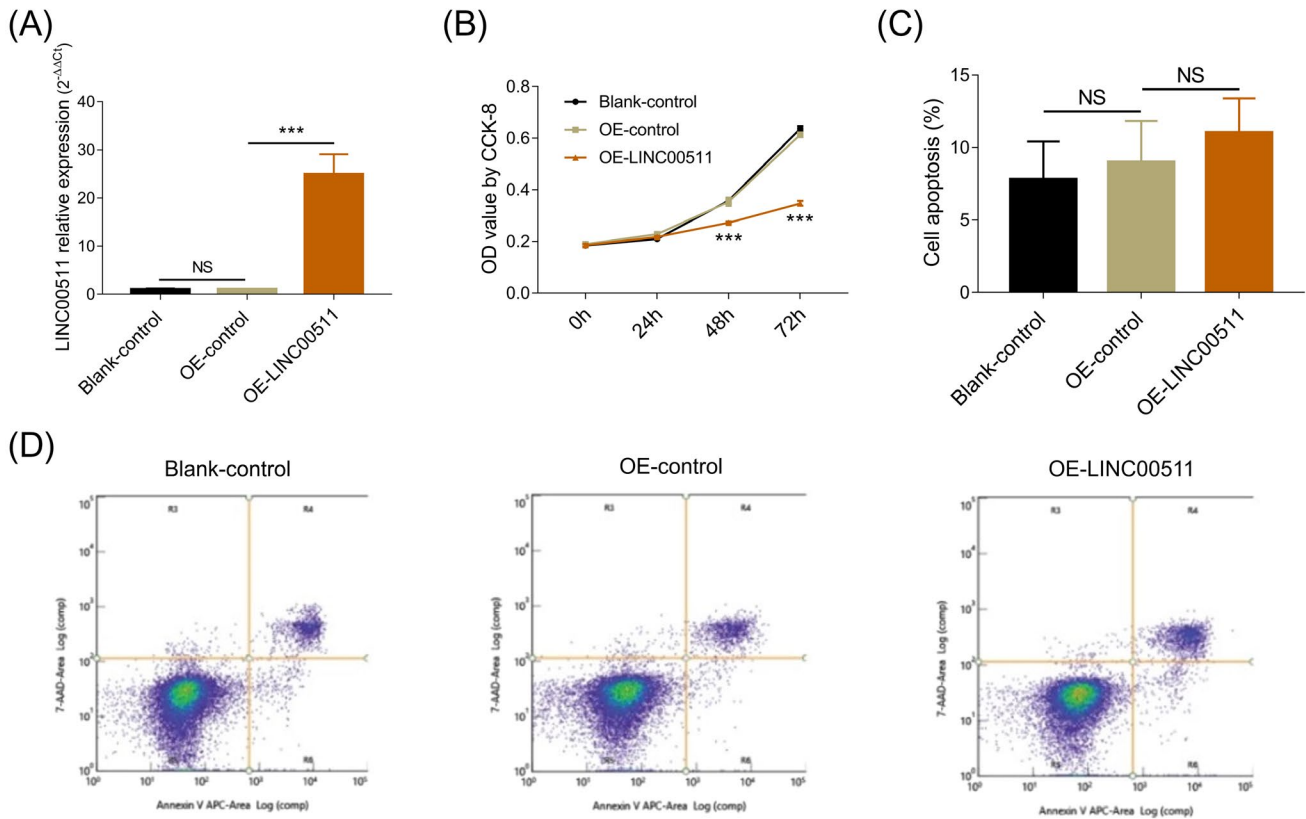


FIGURE 3 LINC00511 expression decreased in osteosarcoma cell lines compared with osteoblast cell line. Comparison of LINC00511 expression among cells was determined by the Dunnett's multiple comparisons test. P value $<.05$ was considered significant. *** $P < .001$. LINC00511: long intergenic non-coding RNA 511

compared with adjacent tissues, which was quite different from these previous studies. In this study, we further disclosed that LINC00511 was correlated with increased TCNR in osteosarcoma patients, which might be caused by that (a) The low expression of LINC00511 might alter the activation of cell survival-related pathways (such as phosphoinositide-3-kinase/protein kinase B (PI3K/Akt) pathway¹⁸) to increase the malignant proliferation of osteoblasts, thereby causing osteosarcoma, therefore, osteosarcoma tissues presented with low expression of LINC00511; (b) LINC00511 suppress cell proliferation, invasion and migration, while increased apoptosis (based on our subsequent experiments) in osteosarcoma cells to decrease malignance of osteosarcoma, therefore increased TCNR in osteosarcoma patients. In this study, the function of LINC00511 in osteosarcoma was different from that in other several cancers reported by the previous studies, which might be caused by different types of cancers and individual variation.

The prognostic value of LINC00511 in several cancers has been investigated in previous studies, which reveal that LINC00511 high expression is correlated with worse prognosis.^{19,20} For example, one previous study reveals that LINC00511 overexpression is correlated with worse OS and it is an independent prognosis factor for OS in patients with liver cancer.¹² Another study discloses that LINC00511 high expression correlates with worse OS in patients with ovarian cancer.¹³ Compared with these previous studies, we discovered the different results that LINC00511 expression was correlated with prolonged OS and LINC00511 high expression was an independent factor that improved OS in patients with osteosarcoma. These results could be explained by that (a) LINC00511 was related to elevated TCNR (above-mentioned), which might increase the effect of neoadjuvant therapy to enhance prognosis in patients with osteosarcoma; (b) LINC00511 might be involved in the alteration of

MG-63 cells



Saos-2 cells

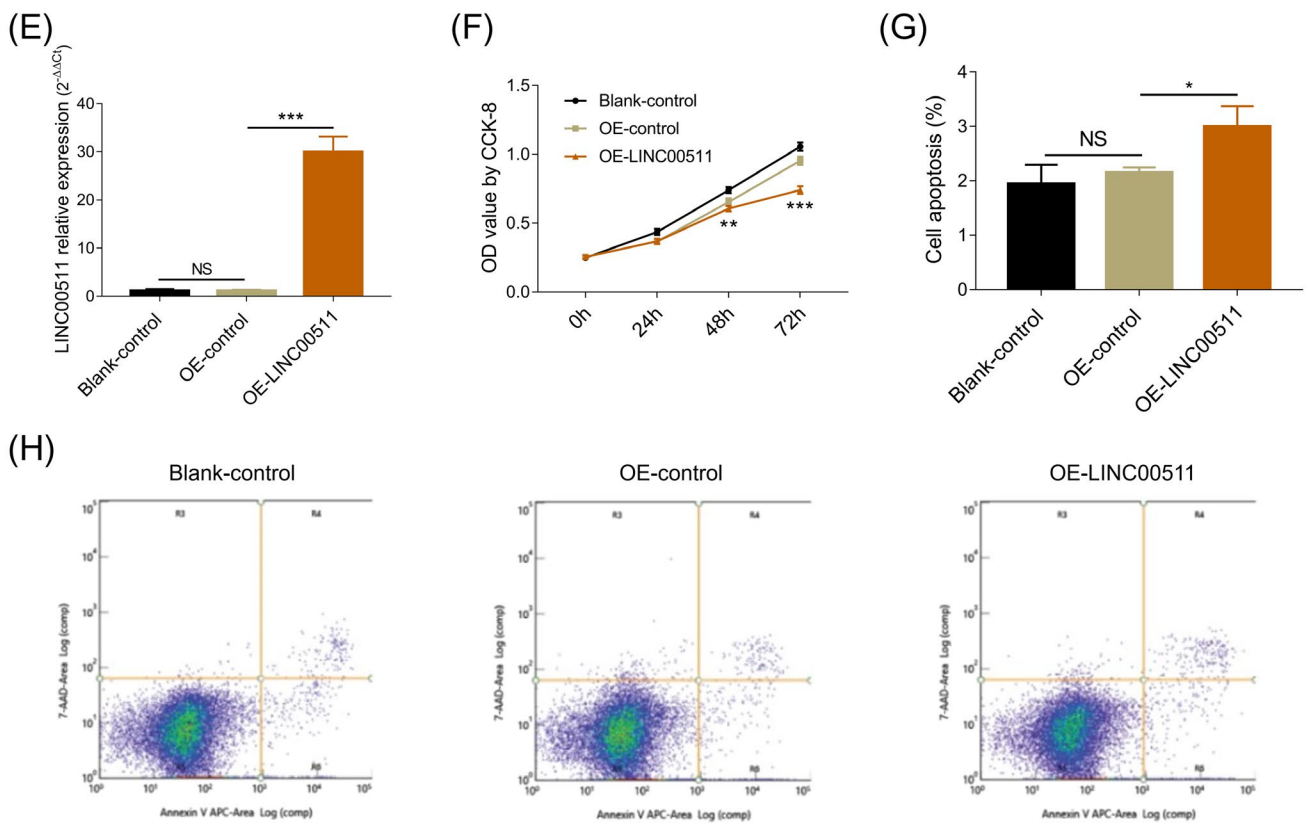


FIGURE 4 LINC00511 suppressed proliferation while increased apoptosis in osteosarcoma cells. A, LINC00511 expression in OE-LINC00511 group, OE-control group and blank-control group in MG-63 cells. B, Cell proliferation in OE-LINC00511 group, OE-control group, and blank-control group in MG-63 cells. C and D, Cell apoptosis in OE-LINC00511 group, OE-control group, and blank-control group in MG-63 cells. E, LINC00511 expression in OE-LINC00511 group, OE-control group, and blank-control group in Saos-2 cells. F, Cell proliferation in OE-LINC00511 group, OE-control group, and blank-control group in Saos-2 cells. G and H, Cell apoptosis in OE-LINC00511 group, OE-control group, and blank-control group in Saos-2 cells. Comparisons of LINC00511 expression, cell proliferation, and cell apoptosis between groups were conducted by Dunnett's multiple comparisons test. P value $<.05$ was considered significant. NS, non-significant; $*P < .05$; $**P < .01$; $***P < .001$. LINC00511: long intergenic non-coding RNA 511; OD, optical density

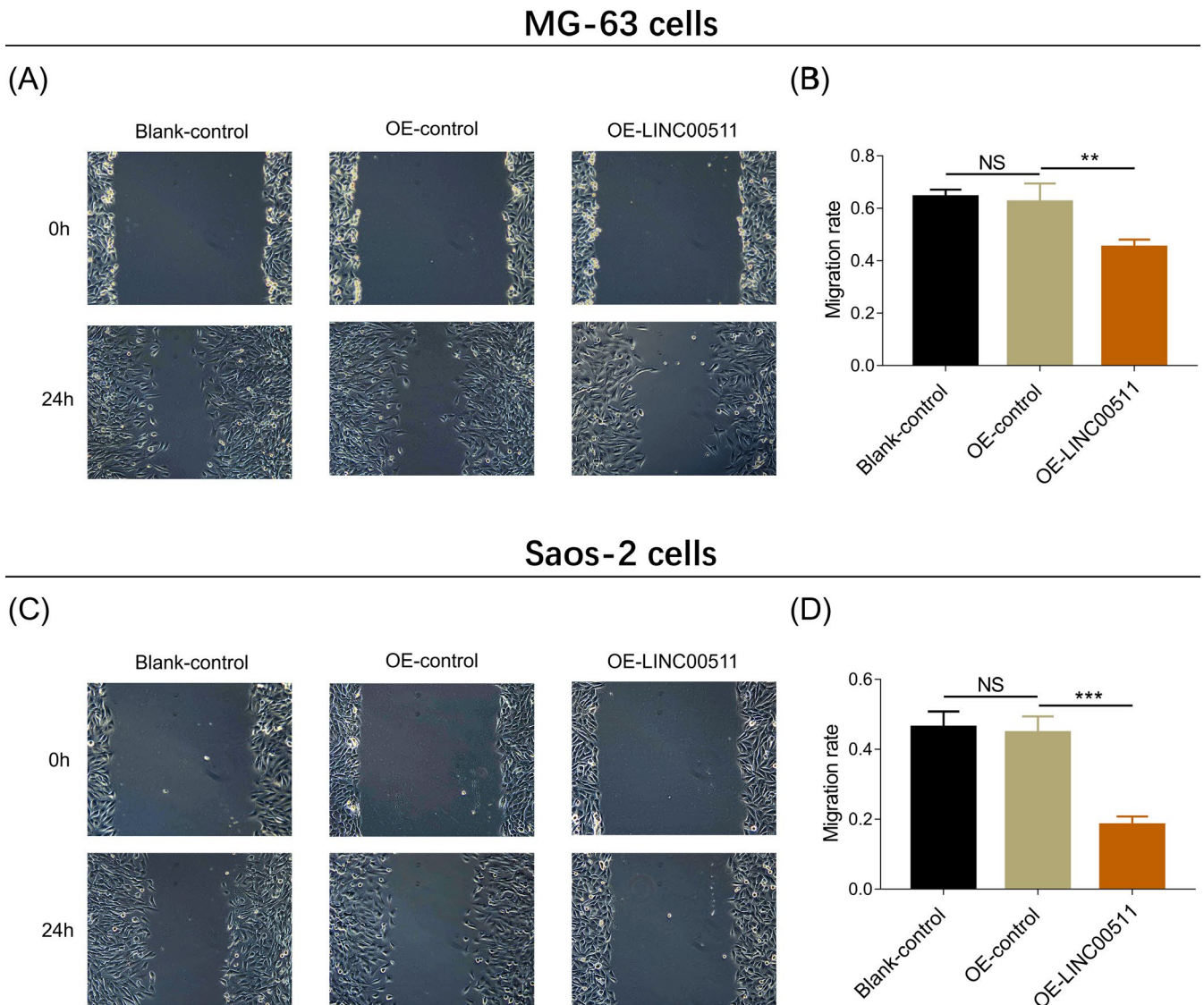
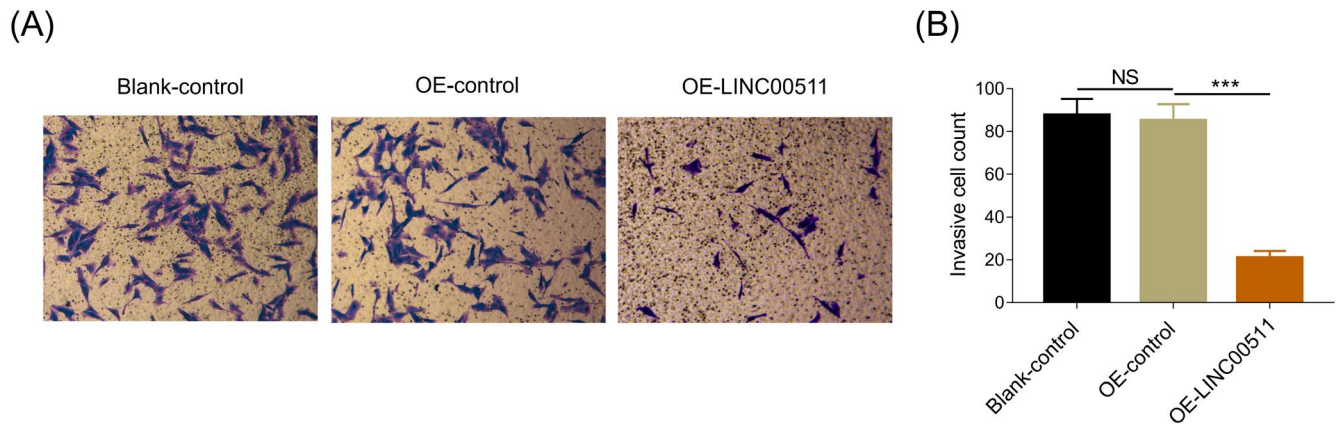


FIGURE 5 LINC00511 repressed cell migration in osteosarcoma cells. A and B, Cell migration rate in OE-LINC00511 group, OE-control group and blank-control group in MG-63 cells. C and D, Cell migration rate in OE-LINC00511 group, OE-control group and blank-control group in Saos-2 cells. Comparison of migration rate between groups was determined by the Dunnett's multiple comparisons test. P value $<.05$ was considered significant. NS, non-significant; $**P < .01$; $***P < .001$. LINC00511: long intergenic non-coding RNA 511

several signaling pathways to inhibit proliferation, migration, and invasion, as well as promote apoptosis in osteosarcoma cells, thus causing reduced tumor progression, and finally resulting in better prognosis in patients with osteosarcoma, which was supported by the following in vitro experiments. Although some interesting results were found, there were still several limitations existing in this study: (a) This study only enrolled osteosarcoma patients without

distant metastasis; therefore, LINC00511 expression and its correlation with OS in patients with distant metastasis were still unclear, and further study enrolling osteosarcoma patients with distant metastasis could be a solution; (b) Osteosarcoma patients with lesion located in the axial bone were not enrolled in this study, and further studies is needed; (c) Since most of the patients were non-endemic, only telephone follow-up was available to record the overall

MG-63 cells



Saos-2 cells

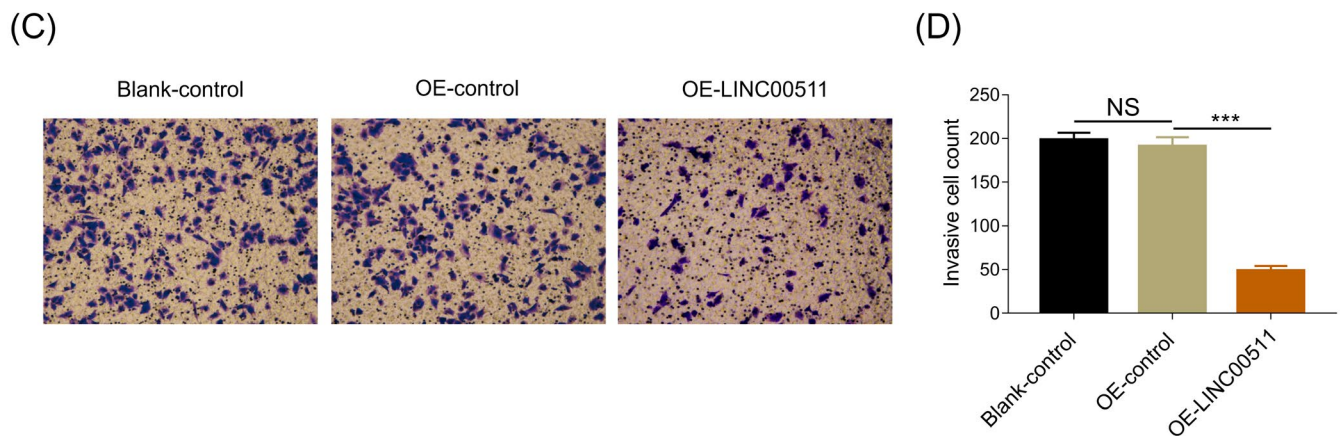


FIGURE 6 LINC00511 inhibited cell invasion in osteosarcoma cells. A and B, Invasive cell count in OE-LINC00511 group, OE-control group and blank-control group in MG-63 cells. C and D, Invasive cell count in OE-LINC00511 group, OE-control group and blank-control group in Saos-2 cells. Comparison of invasive cell count between groups was determined by the Dunnett's multiple comparisons test. P value $<.05$ was considered significant. NS, non-significant; $***P < .001$. LINC00511: long intergenic non-coding RNA 511

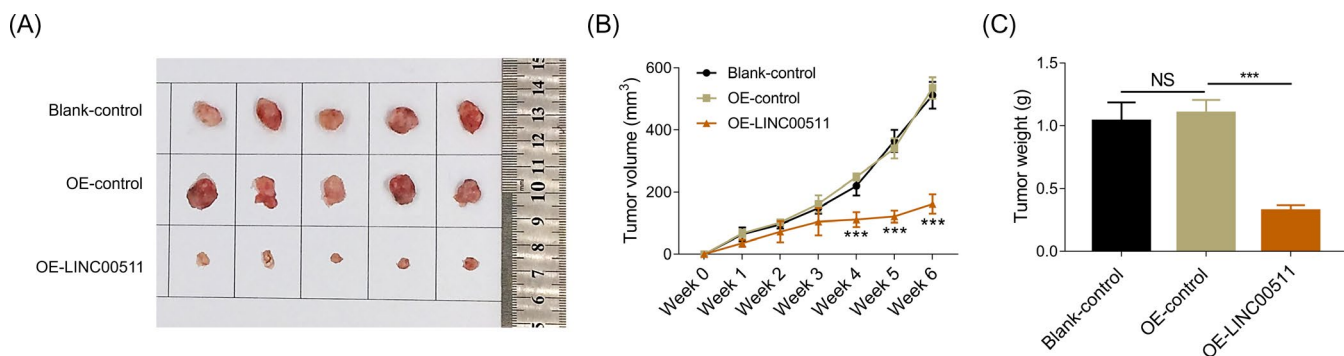


FIGURE 7 LINC00511 decreased tumor progression in xenografted mice. A and B, Tumor volume in OE-LINC00511 group, OE-control group and blank-control group in xenografted mice. C, Tumor weight in OE-LINC00511 group, OE-control group, and blank-control group in xenografted mice. Comparisons of tumor volume and tumor weight between groups were determined by the Dunnett's multiple comparisons test. P value $<.05$ was considered significant. NS, non-significant; $***P < .001$. LINC00511: long intergenic non-coding RNA 511

survival of them, and the recurrence of osteosarcoma was hard to record accurately. Therefore, it was unable to evaluate the correlation of LINC00511 with recurrence-free survival in patients with osteosarcoma.

In order to further investigate the underlying mechanisms of LINC00511 in carcinomas, a few in vitro and in vivo experiments have been performed. For instance, it is reported that LINC00511 could promote proliferation and invasion in tongue squamous carcinoma cells.²¹

Another study reveals that LINC00511 is one of the core genes that influencing MAPK pathway, cell cycle, PI3K/Akt pathway in human epidermal growth factor receptor 2-enriched subtype breast cancer cells using bioinformatics.²² Two studies reported similar results, that knockdown of LINC00511 could reduce tumor size and tumor weight in MDA-MB-231-xenografted nude mice.^{17,23} These studies indicate that LINC00511 acts as an oncogene in several cancers. Whereas the information about the role of LINC00511 in osteosarcoma is still unclear. In this study, we found that LINC00511 expression was decreased in osteosarcoma cells, and LINC00511 overexpression inhibited proliferation, migration, and invasion, as well as promoted apoptosis in osteosarcoma cells. Moreover, we discovered that LINC00511 reduced tumor size and tumor weight in xenografted mice. These results could be explained by that LINC00511 might be involved not only in the regulation of PI3K/Akt pathway, a pathway that promote cell survival, to influence proliferation and apoptosis in osteosarcoma cells, as well as tumor growth in xenografted mice, but also in the alteration of epithelial-mesenchymal transition (EMT) of osteosarcoma cells to affect cell migration and invasion, which should be substantiated in the future.¹⁸ Compared with these previous studies, there were discrepancies about the role of LINC00511 in cancer cell functions, which could be explained by the different types of cancers.

In summary, LINC00511 expression is decreased in tumor tissues, and its elevated expression correlates with increased TCNR and better prognosis in osteosarcoma patients. More significantly, it inhibits osteosarcoma cell survival and motility in vitro, as well as tumor progression in vivo. These data shed light on LINC00511 as a novel biomarker and potential therapeutic option in osteosarcoma.

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ORCID

Zhiwei Wang  <https://orcid.org/0000-0003-2039-2948>

REFERENCES

- Harrison DJ, Geller DS, Gill JD, Lewis VO, Gorlick R. Current and future therapeutic approaches for osteosarcoma. *Expert Rev Anticancer Ther.* 2018;18(1):39-50.
- Moore DD, Luu HH. Osteosarcoma. *Cancer Treat Res.* 2014;162:65-92.
- Picci P. Osteosarcoma (Osteogenic sarcoma). *Orphanet J Rare Dis.* 2007;2:6.
- Briccoli A, Rocca M, Salone M, Guzzardella GA, Balladelli A, Bacci G. High grade osteosarcoma of the extremities metastatic to the lung: long-term results in 323 patients treated combining surgery and chemotherapy, 1985-2005. *Surg Oncol.* 2010;19(4):193-199.
- Isakoff MS, Bielack SS, Meltzer P, Gorlick R. Osteosarcoma: current treatment and a collaborative pathway to success. *J Clin Oncol.* 2015;33(27):3029-3035.
- Mercer TR, Mattick JS. Structure and function of long noncoding RNAs in epigenetic regulation. *Nat Struct Mol Biol.* 2013;20(3):300-307.
- Mercer TR, Dinger ME, Mattick JS. Long non-coding RNAs: insights into functions. *Nat Rev Genet.* 2009;10(3):155-159.
- Cao W, Liu JN, Liu Z, et al. A three-lncRNA signature derived from the Atlas of ncRNA in cancer (TANRIC) database predicts the survival of patients with head and neck squamous cell carcinoma. *Oral Oncol.* 2017;65:94-101.
- Li J, Li Y, Meng F, Fu L, Kong C. Knockdown of long non-coding RNA linc00511 suppresses proliferation and promotes apoptosis of bladder cancer cells via suppressing Wnt/beta-catenin signaling pathway. *Biosci Rep.* 2018;38(4):BSR20171701.
- Xu S, Kong D, Chen Q, Ping Y, Pang D. Oncogenic long noncoding RNA landscape in breast cancer. *Mol Cancer.* 2017;16(1):129.
- Sun CC, Li SJ, Li G, Hua RX, Zhou XH, Li DJ. Long intergenic noncoding RNA 00511 acts as an oncogene in non-small-cell lung cancer by binding to EZH2 and suppressing p57. *Mol Ther Nucleic Acids.* 2016;5(11):e385.
- Wang RP, Jiang J, Jiang T, Wang Y, Chen LX. Increased long non-coding RNA LINC00511 is correlated with poor prognosis and contributes to cell proliferation and metastasis by modulating miR-424 in hepatocellular carcinoma. *Eur Rev Med Pharmacol Sci.* 2019;23(8):3291-3301.
- Wang J, Tian Y, Zheng H, Ding Y, Wang X. An integrated analysis reveals the oncogenic function of lncRNA LINC00511 in human ovarian cancer. *Cancer Med.* 2019;8(6):3026-3035.
- Lu G, Li Y, Ma Y, et al. Long noncoding RNA LINC00511 contributes to breast cancer tumorigenesis and stemness by inducing the miR-185-3p/E2F1/Nanog axis. *J Exp Clin Cancer Res.* 2018;37(1):289.
- Zhao X, Liu Y, Li Z, et al. Linc00511 acts as a competing endogenous RNA to regulate VEGFA expression through sponging hsa-miR-29b-3p in pancreatic ductal adenocarcinoma. *J Cell Mol Med.* 2018;22(1):655-667.
- Li C, Liu H, Yang J, et al. Long noncoding RNA LINC00511 induced by SP1 accelerates the glioma progression through targeting miR-124-3p/CCND2 axis. *J Cell Mol Med.* 2019;23(6):4386-4394.
- Liu R, Wang L, Gan T, Pan T, Huang J, Bai M. Long noncoding RNA LINC00511 promotes cell growth and invasion in triple-negative breast cancer by interacting with Snail. *Cancer Manag Res.* 2019;11:5691-5699.
- Jiang L, Xie X, Ding F, Mei J, Bi R. Silencing LINC00511 inhibits cell proliferation, migration and EMT via PTEN/AKT/FOXO1 signaling pathway in lung cancer. *Biochem Cell Biol.* 2019. <https://doi.org/10.1139/bcb-2018-0364>. [Epub ahead of print].
- Wang W, Lou W, Ding B, et al. A novel mRNA-miRNA-lncRNA competing endogenous RNA triple sub-network associated with prognosis of pancreatic cancer. *Aging (Albany NY).* 2019;11(9):2610-2627.
- Xiao B, Zhang W, Chen L, et al. Analysis of the miRNA-mRNA-lncRNA network in human estrogen receptor-positive and estrogen receptor-negative breast cancer based on TCGA data. *Gene.* 2018;658:28-35.
- Ding J, Yang C, Yang S. LINC00511 interacts with miR-765 and modulates tongue squamous cell carcinoma progression by targeting LAMC2. *J Oral Pathol Med.* 2018;47(5):468-476.
- Yang F, Lyu S, Dong S, Liu Y, Zhang X, Wang O. Expression profile analysis of long noncoding RNA in HER-2-enriched subtype breast cancer by next-generation sequencing and bioinformatics. *Onco Targets Ther.* 2016;9:761-772.
- Zhang J, Sui S, Wu H, et al. The transcriptional landscape of lncRNAs reveals the oncogenic function of LINC00511 in ER-negative breast cancer. *Cell Death Dis.* 2019;10(8):599.

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