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# Prognostic value of long non-coding RNA HOST2 expression and its tumor-promotive function in human osteosarcoma

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**Abstract. – OBJECTIVE: Aberrant expression of long noncoding RNA (lncRNA) is associated with carcinogenesis of various tumors. The aim of current study was to explore the clinical significance and biological function of long non-coding RNA HOST2 (lnc-HOST2) in patients with osteosarcoma.**

**PATIENTS AND METHODS: The expression of lnc-HOST2 was detected by quantitative Real-time PCR (qRT-PCR) in 163 osteosarcoma specimens and matched the normal tissues. The significance of lnc-HOST2 as a prognostic factor as well as its relationship with survival was determined. The effects of lnc-HOST2 expression on the biological behavior of osteosarcoma cells were investigated by MTT and flow cytometry. qRT-PCR and Western blot were used to evaluate the mRNA and protein expression of apoptosis-related factors.**

**RESULTS: The expression levels of lnc-HOST2 in osteosarcoma tissues were significantly higher than those in corresponding noncancerous bone tissues ($p < 0.01$). Statistical assay indicated that the expression level of lnc-HOST2 was positively correlated with tumor stage ($p = 0.003$) and distant metastasis ($p = 0.000$). Furthermore, Kaplan-Meier analysis suggested that patients with high lnc-HOST2 showed poorer overall survival than those with low lnc-HOST2 ($p = 0.000$). The univariate and multivariate analysis further revealed that lnc-HOST2 expression was an independent prognostic factor for overall survival. Then, functionally, down-regulation of lnc-HOST2 suppressed proliferation and induced cell apoptosis in osteosarcoma cells. Notably, we confirmed that up-regulation of lnc-HOST2 led to Bcl-2 downregulation and Bax upregulation in osteosarcoma cells.**

**CONCLUSIONS: We provided the first evidence that lnc-HOST2 may serve as a novel prognostic marker in osteosarcoma. Further, targeting lnc-HOST2 may represent an attractive target therapy for osteosarcoma.**

*Key Words:*

Long noncoding RNA, Osteosarcoma, Prognosis, Proliferation.

## Introduction

Osteosarcoma is a primary mesenchymal tumor with invasive growth characteristics, and it can occur at any age, but most patients are diagnosed between the age of 10 and 25 years[1,2]. Advances in osteosarcoma therapy over the past decades have enhanced patient prognosis, the 5-year survival for osteosarcoma patients has increased to 60-70%[3]. Major reasons for high mortality rate in osteosarcoma are metastasis or invasiveness rather than primary tumour lesions[4]. Thus, characterization and investigation of novel osteosarcoma biomarkers which were associated with treatment and prognosis are urgently needed to improve the treatment of this disease.

Long non-coding RNAs (lncRNAs) are a class of noncoding RNAs which are greater than 200 nucleotides in length and have no protein-coding capacity[5]. Increasing evidence reveals that cancer lncRNAs may mediate oncogenic or tumor-suppressing effects by affecting cell proliferation, migration, immune response, and apoptosis[6-8]. The dysregulation of lncRNAs has also been reported to contribute to the initiation and progression of various tumors, including osteosarcoma[9]. For instance, Wang et al[10] found that forced expression of lncRNA LeXis could promote osteosarcoma growth through upregulation of CTNNB1 expression. Cui et al[11] reported that high lncRNA HOXA11-AS was associated with advanced clinical stage and distant metastasis in osteosarcoma patients, and its silencing inhibits cell proliferation and invasion by sponging miR-124-3p in osteosarcoma. However, the role of lncRNAs in osteosarcoma is still in its infancy, and most lncRNAs have not been identified.

The novel gene lncRNA human ovarian cancer-specific transcript 2(lnc-HOST2), which is located on 10q23.1, is one of the most up-regulated genes in epithelial ovarian cancer[12]. Recently

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Wang et al[13] showed that lnc-HOST2 served as a tumor promoter in osteosarcoma. Thus, we hypothesized that the dysregulation of lnc-HOST2 may be associated with prognosis of osteosarcoma patients. In this study, we aimed to explore the prognostic value of lnc-HOST2 and confirm its biological function by *in vitro* assay.

## Patients and Methods

### Patients and Tissue Samples

One hundred and sixty-three osteosarcoma tissue samples and matched adjacent non-cancer tissues were selected from patients who underwent surgery between 2006 and 2011 at Department of Orthopaedics, Shanghai Eighth People's Hospital. A total of 98 males and 65 females were enrolled in this investigation, and the median age was 29 years (range, 12-45). None of the patients had received radiotherapy or chemotherapy before surgery. All cancerous and tissue specimens and paired normal bone tissues were histologically confirmed by two pathologists and snap-frozen in liquid nitrogen. Tumor tissue samples were grouped according to the sixth edition of the TNM classification of the International Union against Cancer (UICC). All patients gave their informed consent and protocols were approved by Institutional Ethics Committees of Shanghai Eighth People's Hospital.

### Cell Lines and Transfection

The human osteosarcoma cell lines (U2OS, MG-63, and KHOS) and human normal osteoblast cell line NHost were purchased from ATCC (Manassas, VA, USA). Cells were maintained in Dulbecco's Modified Eagle Medium (DMEM; GibCo BRL, Grand Island, NY, USA) supplemented with 10% heat-inactivated fetal bovine serum and 100 units/ml penicillin/streptomycin at 37°C in a humidified atmosphere of 5% $CO^2$.

Osteosarcoma cell lines were transfected with 50 nM lnc-HOST2 siRNA (si-lnc-HOST2) (si-lnc-HOST2: GACUAAACAAGGUCUUAAUTT) and negative control siRNA (si-NC) using Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA). The cells were harvested 48 h after transfection.

### RNA Isolation and Real-time-PCR

Total RNA was extracted using TRIzol according to the manufacturer's protocol. qRT-PCR assays were performed to detect lnc-HOST2 expression using the Prime Script RT reagent Kit and SYBR Premix ExTaq (TaKaRa, Otsu, Shiga, Japan). The primer sequences were shown in Table I. The primers for Bcl-2 and Bax mRNA was purchased from Invitrogen (Carlsbad, CA, USA). Lnc-HOST2 relative expression was calculated and normalized using the $2^{-\Delta\Delta Ct}$ relative to GAPDH. Independent experiments were done in triplicate.

### Cell Proliferation Assay

24 h after transfection, cells were plated into 96 well plate at a density of 3000 cells/well. At a series of time points, 20 mL of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) was added to each well, and the cells were then incubated at 37°C for 4 h. Subsequently, the supernatant was replaced by 150 μl of dimethyl sulfoxide to dissolve the formazan crystals. OD490 (nm) value in each well was determined by a microplate reader. Each assay was repeated three independent times in triplicate.

### Apoptosis Assay

The cancer cells were harvested and resuspended in 500 μl of a binding buffer. The cells were washed with phosphate-buffered saline (PBS) and, then, stained with 5 μl of Annexin V and 5 μl of propidium iodide (PI). After incubation, the cells were left at room temperature in the dark for 15 min. Samples were then analyzed via flow cytometry.

### Western Blot Assays

Western blot was carried out[14]; in brief, the interest protein was resolved by sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to a polyvinylidene difluoride PVDF (membrane). The membranes were then consecutively incubated with specific primary antibodies and specific secondary antibodies after blocked with 5% (v/v) bovine serum albumin (BSA). The immunoreactive proteins were detected by ECL Detection Systems (ThermoFisher Scientific, Waltham, MA, USA).

**Table I.** The primer sequence of lnc-HOST2 and GAPDH.

| Gene | Sequence |
|---|---|
| lnc-HOST2 | F: 5'-CTCAAATCAATCACGACCCT-3'<br>R: 5'-AATGTAGCAGGACGAGCC-3'; |
| GAPDH | F: 5'-GTCAACGGATTTGGTCTGTATT-3'<br>R: 5'-AGTCTTCTGGGTGGCAGTGAT-3' |

### Statistical Analysis

All analyses were performed using the SPSS 19.0 statistical software package (SPSS Inc., Chicago, IL, USA). The significance of differences between groups was estimated by the Student's *t*-test and the chi-square test. Survival data were computed using the Kaplan-Meier method and compared between groups by the log-rank test. The significance of survival variables was analyzed using the Cox multivariate proportional hazards model. A two-sided probability value of less than 0.05 was considered statistically significant.

## Results

### High Expression of lnc-HOST2 in Osteosarcoma Tissues and Cell Lines

Firstly, we analyzed the expression level of lnc-HOST2 in osteosarcoma tissues and matched normal bone tissues. Significantly, the relative expression of lnc-HOST2 in osteosarcoma tissues was significantly higher than in adjacent normal tissues (Figure 1A, $p < 0.01$). Then, we further analyzed the expression level of lnc-HOST2 in NHost and three human osteosarcoma cell lines including U2OS, MG-63, and KHOS by qRT-PCR. Significantly, the expression level of lnc-HOST2 was significantly higher in osteosarcoma cells lines than in NHost cells (Figure 1B, $p < 0.01$). This result suggested lnc-HOST2 might act as a tumor promoter in osteosarcoma.

### Relationship Between lnc-HOST2 Expression and Clinicopathological Variables in Osteosarcoma Patients

The patients were divided into high lnc-HOST2 expression group and low lnc-HOST2 expression group according to the equal expression of lnc-HOST2. Table II listed the relationship between lnc-HOST2 expression and the clinicopathological parameters. The expression level of lnc-HOST2 was positively correlated with tumor stage ($p = 0.003$) and distant metastasis ($p = 0.000$). However, the lnc-HOST2 expression level was not associated with other parameters such as age, gender, tumor site and differentiation status ($p > 0.05$).

### Significance of lnc-HOST2 Expression in Osteosarcoma Prognosis

Then, we explored the prognostic value of the lnc-HOST2 expression in patients with osteosarcoma. Overall survival curves in the high lnc-HOST2 group and low the lnc-HOST2 group were shown in Figure 2. As was expected, patients with higher lnc-HOST2 expression had a shorter overall survival time than those with lower lnc-HOST2 expression ($p = 0.000$). Then, the univariate analysis demonstrated that tumor stage ($p = 0.004$), distant metastasis ($p = 0.001$) and lnc-HOST2 expression ($p = 0.001$) were significantly correlated with overall survival of osteosarcoma patients (Table III). With multivariate analysis, we confirmed lnc-HOST2 as an independent prognostic factor ($p = 0.002$), with 95% confidence interval 1.293-4.772 and HR 2.352 (Table III).

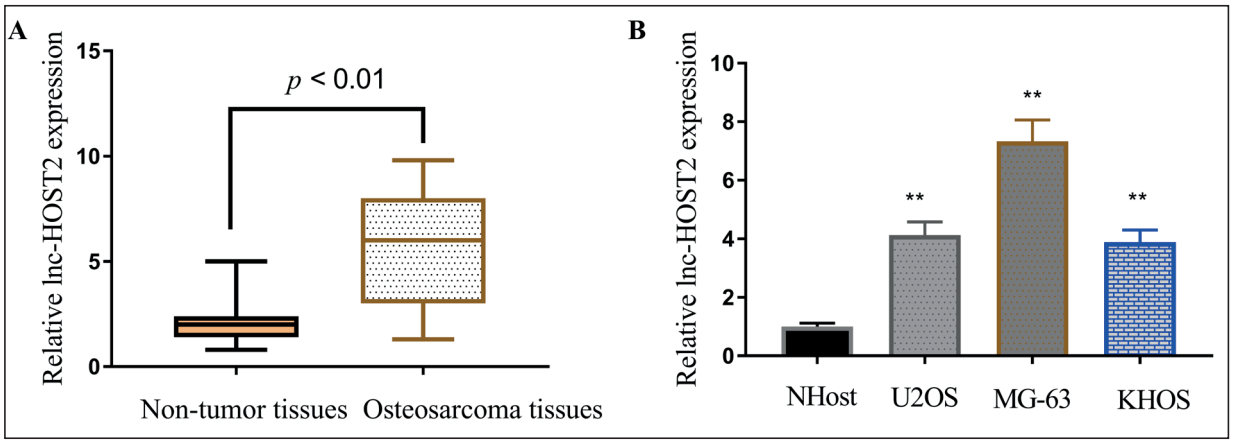


**Figure 1.** qRT-PCR of lnc-HOST2 in human osteosarcoma clinical tissues and cell lines. ***A,*** Relative expression of lnc-HOST2 in human osteosarcoma tissues and corresponding nontumor tissues. ***B,*** Relative expression of lnc-HOST2 in osteoblast cell line (NHost) and three osteosarcoma cell lines (U2OS, MG-63 and KHOS). *$p < 0.05$, **$p < 0.01$.

**Table II.** Correlation between relative lnc-HOST2 expression and clinicopathological characteristics in osteosarcoma (n = 163).

| Characteristics | Cases (163) | lnc-HOST2 expression | | *p*-value |
|---|---|---|---|---|
| | | High | Low | |
| Age (years) | | | | 0.639 |
| ≤ 20 | 73 | 33 | 40 | |
| > 20 | 90 | 44 | 46 | |
| Gender | | | | 0.235 |
| Male | 98 | 50 | 48 | |
| Female | 65 | 27 | 38 | |
| Tumor site | | | | 0.489 |
| Femur/tibia | 123 | 60 | 63 | |
| Others | 40 | 17 | 23 | |
| Differentiation status | | | | 0.132 |
| High | 65 | 26 | 39 | |
| Low | 98 | 51 | 47 | |
| Tumor stage | | | | 0.003 |
| I + II | 79 | 28 | 51 | |
| III | 84 | 49 | 35 | |
| Distant metastasis | | | | 0.000 |
| Yes | 83 | 53 | 30 | |
| No | 80 | 24 | 56 | |

### *Down-regulation of lnc-HOST2 Reduces Cell Viability and Promotes Cell Apoptosis*

To further study the function of lnc-HOST2 in osteosarcoma cell proliferation, U2OS cells stably down expressing lnc-HOST2 were established and relative lnc-HOST2 expression was determined using real-time PCR (Figure 3A). MTT assay showed that down-regulation of lnc-HOST2 significantly decreased the growth rate of U2OS cell (Figure 3B). Moreover, we performed flow cytometer to explore the effect of lnc-HOST2 in osteosarcoma cell apoptosis. As shown in Figure 3C, we found that the percentage of apoptotic U2OS cells was significantly elevated after down-regulation of lnc-HOST2. Then, we further explored the effect of lnc-HOST2 on the expression of Bcl-2 and Bax. As shown in Figure 3D, the results of PCR showed that down-regulation of lnc-HOST2 led to Bcl-2 mRNA downregulation and Bax mRNA upregulation in U2OS cells ($p < 0.01$). Moreover, the results of Western blot also indicated that down-regulation of lnc-HOST2 led to Bcl-2 proteins downregulation and Bax proteins upregulation in U2OS cells (Figure 3E, $p < 0.01$). Thus, our findings revealed that down-regulation of lnc-HOST2 suppressed cell proliferation and induced apoptosis in osteosarcoma cells.

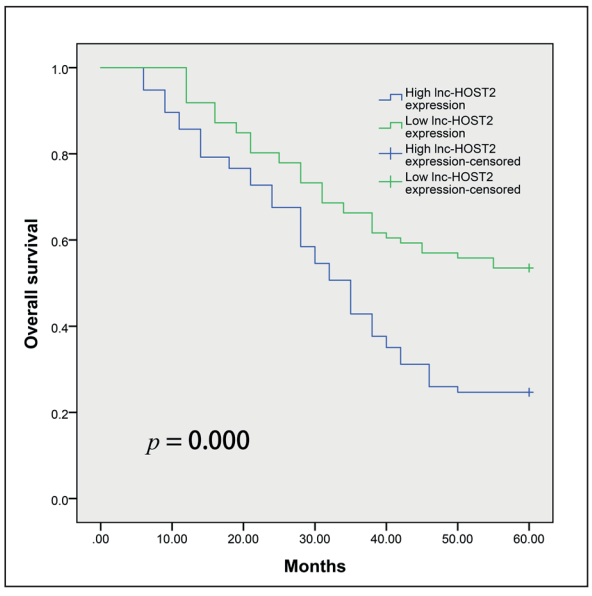


**Figure 2.** The expression of lnc-HOST2 in relation to overall survival in the patients with osteosarcoma.

## Discussion

Identifying reliable molecular biomarkers for osteosarcoma are crucial to the clinical management of osteosarcoma because they can offer guidance for risk stratification and treatment decision[15]. Although several prognostic factors for patients with osteosarcoma have been identified, such as age, sex, differentiation status, and tumor size, the accuracy of these factors is not satisfac-

**Table III.** Univariate and multivariate analysis of overall survival in osteosarcoma patients.

| Variables | Univariate analysis | | | Multivariate analysis | | |
|---|---|---|---|---|---|---|
| | HR | 95% CI | *p* value | HR | 95% CI | *p* value |
| Age | 1.423 | 0.733-1.932 | 0.421 | – | – | – |
| Gender | 1.263 | 0.849-2.142 | 0.216 | – | – | – |
| Tumor site | 1.738 | 0.716-1.664 | 0.255 | – | – | – |
| Differentiation status | 1.622 | 0.813-2.213 | 0.134 | – | – | – |
| Tumor stage | 3.321 | 1.562-5.621 | 0.004 | 2.983 | 1.328-4.273 | 0.007 |
| Distant metastasis | 4.231 | 1.831-8.558 | 0.001 | 3.892 | 1.558-7.132 | 0.001 |
| lnc-HOST2 expression | 2.787 | 1.432-5.348 | 0.001 | 2.352 | 1.293-4.772 | 0.002 |

tory[16,17]. Fortunately, growing evidence indicated that lncRNAs would be ideal prognostic molecular biomarkers and therapeutic targets in osteosarcoma in the future[18,19].

As is known, lncRNAs participated in the biological function of cancer cells[20]. The biological function of lnc-HOST2 has been reported in several tumors. For instance, Peng et al[21] reported that up-regulation of lnc-HOST2 promoted ovarian cancer cell migration and invasion by inhibiting let-7. Liu et al[22] showed that the increased expression of lnc-HOST2 in hepatocellular carcinoma was correlated with distant metastasis, TNM staging, and differentiation degree, and knockdown of lnc-HOST2 reduced migration and invasion abilities of hepatocellular carcinoma cells. Wang et al[13] found that the expression levels of lnc-HOST2 were significantly up-regulated in osteosarcoma tissues and cell lines. Moreover, *in vitro* assay indicated that lnc-HOST2 inhibition could inhibit the proliferation, migration, and invasion. Those results suggested lnc-HOST2 as an oncogene in several tumors, including osteosarcoma. However, the complex molecular mech-

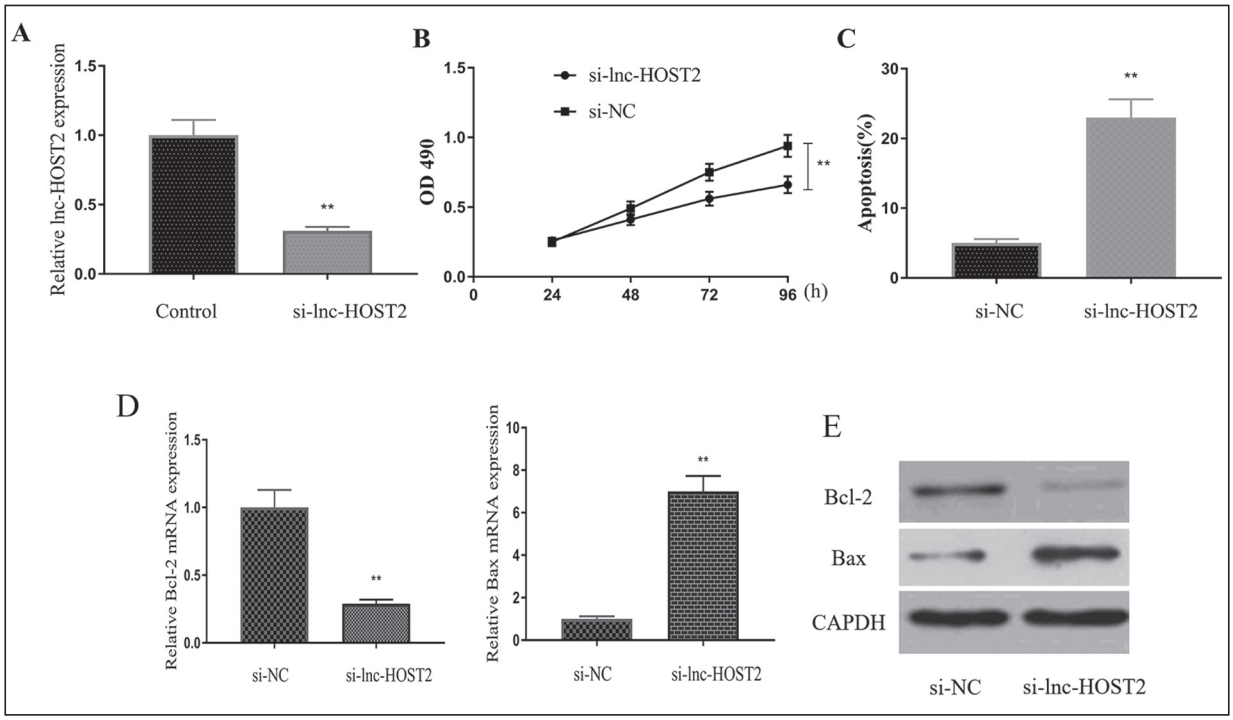


**Figure 3.** Down-regulation of lnc-HOST2 decreased cell proliferation and promoted apoptosis in osteosarcoma cells via affecting Bcl-2 and Bax. ***A,*** The expression of lnc-HOST2 was decreased after treated with si-lnc-HOST2. ***B,*** Cell proliferation was determined in U2OS cells transfected with si-lnc-HOST2 or si-NC by MTT assay. ***C,*** Rate of apoptosis cell population was counted by flow cytometry. ***E,*** The expression of Bcl-2 mRNA and Bax mRNA after treated with si-lnc-HOST2 by qRT-PCR. ***F,*** The expression of Bcl-2 and Bax protein after treated with si-lnc-HOST2 by Western blot. *$p < 0.05$, **$p < 0.01$.

anisms underlying high lnc-HOST2 expression in lnc-HOST2 and its prognostic value are still incompletely known.

In the present work, we reported that lnc-HOST2 was prominently upregulated in osteosarcoma tissues and cell lines. These findings were in line with the previous report[13]. Then, we investigated the correlation between the expression of lnc-HOST2 and clinicopathological features of osteosarcoma. The results showed that high lnc-HOST2 expression was positively correlated with tumor stage and distant metastasis. Kaplan-Meier analysis revealed that glioma patients with high lnc-HOST2 expression had the poorest overall survival. This suggests that lnc-HOST2 plays a potential role in bone carcinogenesis. Moreover, by the Cox proportional hazards model, we confirmed that lnc-HOST2 expression was an independent prognostic factor for patients with osteosarcoma.

To explore the biological function of lnc-HOST2 in osteosarcoma progression, we further performed *in vitro* to explore the effect of lnc-HOST2 on proliferation and apoptosis in osteosarcoma cells. Based on our data, we confirmed that down-regulation of lnc-HOST2 signally inhibited growth and facilitated apoptosis. Next, we performed Western blot to detect the expression levels of apoptosis-related proteins and found that down-regulation of lnc-HOST2 led to Bcl-2 proteins downregulation and Bax proteins upregulation. Our results confirmed the tumor promoting role of lnc-HOST2 in osteosarcoma. However, a further understanding of the molecular mechanism by lnc-HOST2 in human osteosarcoma needed.

## Conclusions

Our study demonstrated for the first time that lnc-HOST2 dysregulation was associated with osteosarcoma clinical features and poor survival rates. Furthermore, we revealed that down-regulation of lnc-HOST2 suppressed proliferation and promoted cell apoptosis by affecting the expression of Bcl-2 and Bax. Overall, our data suggested that lnc-HOST2 may serve as a new prognostic biomarker and therapeutic target of osteosarcoma.

### Conflict of Interest

The Authors declare that they have no conflict of interests.

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