Original Paper

Effects of the Long Non-Coding RNA HOST2 On the Proliferation, Migration, Invasion and Apoptosis of Human Osteosarcoma Cells

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Key Words

Long non-coding RNA-HOST2 • Osteosarcoma • Proliferation • Migration • Invasion • Apoptosis

Abstract

Background/Aims: This study aimed to explore the effects of the long non-coding RNA HOST2 (lnc-HOST2) on the proliferation, migration, invasion and apoptosis of osteosarcoma cells. **Methods:** Osteosarcoma tissues and adjacent normal tissues from 52 patients were selected. Human osteosarcoma cell lines (SaOS2, HOS, U2OS and MG-63) were collected and cultured; MG-63 cells had the highest lnc-HOST2 expression and thus were used in subsequent experiments. Then, MG-63 cells were transfected and divided into the blank (no transfection), si-CON (transfected with negative control siRNA) and si-lnc-HOST2 (transfected with small interference lnc-HOST2 siRNA) groups. Quantitative real-time polymerase chain reaction (qRT-PCR) was used to determine the expression of lnc-HOST2 in primary tissues and cells. Cell growth was detected using the CCK-8 and colony formation assays. Cell doubling time was detected. Cell migration and invasion were observed using the scratch test and Transwell assays. Cell apoptosis and cell cycle progression of osteosarcoma cells were detected using flow cytometry with annexin V/PI double staining and PI staining, respectively. **Results:** The level of lnc-HOST2 expression in the si-lnc-HOST2 group was significantly decreased compared to that in the blank and si-CON groups. The OD values in the si-lnc-HOST2 group were significantly lower than those in the blank and si-CON groups. Compared to the blank and si-CON groups, the si-lnc-HOST2 group presented significant decreases in the colony number and healing rates after scratching. The number of invasive cells in the si-lnc-HOST2 group was significantly less than that in the blank and si-CON groups. In the si-lnc-HOST2 group, the cell cycle was mainly halted in the G1 phase, and the apoptosis rate and doubling time in this group were significantly higher than those in the blank group and si-CON group. **Conclusions:** Inhibition of lnc-HOST2 could suppress the proliferation, migration, and invasion and promote the apoptosis of osteosarcoma cells.

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Introduction

Osteosarcoma is a primary malignant tumor of the skeleton characterized by the direct formation of immature bone or osteoid tissue by tumor cells. Osteosarcoma rarely arises in soft tissue [1]. Osteosarcoma is the most common primary tumor of the bone that typically affects the long tubular bones of children and adolescents. The prognosis of patients with high-grade osteosarcoma who are treated with surgery alone has been very poor with 5-year survival rates below 20% [2]. The age distribution of osteosarcoma is the greatest during the skeletal growth period of adolescence [3]. In these years, the 5-year survival rate was only 30-40% despite the use of surgery, and radiation combined with adjuvant chemotherapy, [4]. Therefore, finding advanced diagnostics and therapies for osteosarcoma is critical for future studies. Many reports have described the development of tumors with regard to non-coding RNAs [5].

Long non-coding RNAs (lncRNAs) are RNA molecules that are longer than 200 nt in length and lack an open reading frame. Recent studies have provided evidence that lncRNAs play significant roles in a variety of cellular processes via interactions with proteins in gene regulatory systems; additionally, alterations in their cell- or tissue-specific expression and/or their primary or secondary structures are thought to promote cell proliferation, metastasis and invasion [6]. With the advances of high-resolution arrays and massive parallel sequencing technologies, lncRNAs have gained attention to date and have been found to play important roles in the diagnosis and treatment of many disease, especially in the tumorigenesis and progression of human cancers, such as human non-small cell lung cancer and papillary thyroid cancer [9]. Moreover, increasing evidence has proven to support the notion that lncRNAs can be used in plasma as biomarkers for the diagnosis of multiple diseases [10-12]. Rang et al. [11] that database analyses, cloning and sequencing of the corresponding expressed sequence tags provided details regarding some of these transcripts, namely, human ovarian cancer-specific transcripts (HOSTs). As a novel gene, HOST2 contains multiple copies of retroviral-related sequences in the absence of an obvious open reading frame [12]. HOST2 can promote tumor cell proliferation, migration, and invasion in epithelial ovarian cancer, exerting its functions in key aspects of biological behaviors, and a bioinformatic analysis revealed that HOST2 could bind the potent tumor suppressor microRNA let-7, which was verified to target HOST2 [14]. lncRNAs may be used as novel candidate biomarkers for the clinical diagnosis and treatment of some diseases and could serve as potential targets for future therapies [12]. However, lncRNA-HOST2 has not been characterized in human osteosarcoma samples to date. In this study, we aimed to investigate the effect of lnc-HOST2 on the proliferation, migration, invasion and apoptosis of osteosarcoma cells.

Materials and Methods

Ethnic subjects

This experiment was approved by the Ethics Committee at Linyi People's Hospital. All patients signed informed consent.

Study subjects

Between January 2008 and January 2014, osteosarcoma tissues and adjacent normal tissues (at least 5 cm away from the tumor site) were obtained from 52 osteosarcoma patients who underwent surgery at Linyi People's Hospital. Tissue samples were immediately frozen in liquid nitrogen for further use. There were 27 females and 25 males, and the median age of the cohort was 15.2 years old. All the osteosarcoma patients were confirmed by postoperative pathological examinations. The histological types of the 52 samples contained osteogenic tissue (n = 25), cartilaginous tissue (n = 16), and fibrous tissue (n = 11).

Cell culture and selection

The osteosarcoma SaOS2, HOS and U2OS cell lines were purchased from American Type Culture Collection (ATCC) and cultured in Dulbecco's Modified Eagle's medium (DMEM) (Gibco, Gaithersburg, MD,

USA) with 10% fetal bovine serum (FBS) (HyClone, USA). The osteosarcoma MG-63 cell line (purchased from Shanghai cell bank) was cultured in 1640 medium (Gibco, Gaithersburg, MD, USA) with 10% FBS. Cells were incubated at 37 °C in an atmosphere containing 5% CO₂ to approximately 70% confluence and then passaged. After removal of the original medium, the cells were rinsed twice with phosphate-buffered saline (PBS), digested in 0.25% trypsin (Gibco, Gaithersburg, MD, USA), and resuspended in DMEM or 1640 medium (as appropriate) containing 10% FBS to form a single-cell suspension for routine passages. Cells in the logarithmic growth phase were used for our experiment.

Cell transfection and grouping

MG-63 cells in the logarithmic growth phase were digested, counted, and seeded in a 96-well plate at a density of 2×10^5 cells per well to approximately 50% ~ 60% confluency. Then, the cells were transiently transfected with Lipofectamine 2000 according to the manufacturer's instructions (Invitrogen, Carlsbad, CA, USA). The cells were transfected with either negative control siRNA or 50 nmol/L lnc-HOST2 siRNA (purchased from Shanghai Gene Pharma Co., Ltd, China). The transfected cells were incubated at 37 °C with 5% CO₂. After 4 to 6 hours, the transfection solution was replaced with fresh culture medium. Culture medium in the wells was removed and replaced with 1640 medium containing 10% FBS for subsequent experiments. Cells in the wells were cultivated for another 24 to 48 hours. The cells were divided into three groups: the blank (without any transfection), si-CON (transfected by negative control siRNA), and si-lnc-HOST2 (transfected by small interference lnc-HOST2 siRNA) groups. The sense sequence of the lnc-HOST2 siRNA was 5'-GACUAAACAAGGUCUUAUUT-3', and the antisense sequence was 5'-AAACCUUGUUUAGUTCC-3'. The sense sequence of the negative control siRNA was 5'-UUCUCCGAACGUGUCACGUTT-3', and the antisense sequence was 5'-ACGUGACACGUUCGGAGAATT-3'. Meanwhile, a GAPDH siRNA group was used as a positive control with the sense sequence 5'-GUAUGACUUAUUAUUAAGT-3' and the antisense sequence 5'-CUUGAGGCUGUUGUCAUACTT-3' [15]. These sequences were designed and constructed by GenePharma Co., Ltd. (Shanghai, China). In addition, we conducted flow cytometry to detect the transfection efficiency, which indicated that among 2.0×10^5 cells per well, the transfection efficiency was 97.3%, and 70% of the signal was detected.

Quantitative real-time polymerase chain reaction (RT-PCR)

Total RNA was extracted from MG-63 cells (from all the groups 48 hours after transfection) using an miRNeasy Mini Kit (Qiagen, Crawley, UK). The concentration and purity of RNA were detected by measuring the ultraviolet absorbance at 260 nm and 280 nm (optical density, OD260/OD280 ratio) using an ultraviolet spectrophotometer. An OD260/OD280 ratio between 1.7 and 2.1 indicated that the RNA had high purity for subsequent experiments. The cDNA template was synthesized using reverse transcription with the PrimeScript RT-PCR system (ABI). The qRT-PCR experiment was conducted using an ABI 7500 Real-Time PCR system. The reaction conditions were as follows: initial denaturation at 95 °C for 10 min followed by 40 cycles of denaturation at 95 °C for 10 s, annealing at 60 °C for 20 s, and extension at 72 °C for 34 s. The reaction system comprised 10 µl of SYBR Premix Ex Taq™ II, 0.8 µl of PCR Forward Primer (10 µM), 0.8 µl of PCR Reverse Primer (10 µM), 0.4 µl of ROX Reference Dye, 2.0 µl of cDNA template, and 7.6 µl of sterile distilled water. According to the full-length gene sequence of HOST2 lncRNA, specific primers for HOST2 lncRNA were designed using Primer 5, and reference primers were used as previously established [16]. The primer sequences (synthesized by Shanghai Invitrogen Biotechnology Co., Ltd., Shanghai, China) were as follows: forward primer for lnc-HOST2, 5'-CTCAAATCAATCAGACCCT-3'; reverse primer for lnc-HOST2 was 5'-AATGTAGCAGGACGAGCC-3'; forward primer for the reference gene GAPDH, 5'-ACCCATGGCAAATTCATGGCA-3'; reverse primer for GAPDH, 5'-TCTAGACGGCAGGTCAGGTCCACC-3'. Using GAPDH gene as a reference, the relationship between the expression of the target gene in the experimental group and the control group was expressed using the $2^{-\Delta\Delta Ct}$ method ($\Delta\Delta Ct = \Delta Ct_{\text{experimental group}} - \Delta Ct_{\text{control group}}$, $\Delta Ct = Ct_{\text{lnc-HOST2}} - Ct_{\text{GAPDH}}$, where Ct is the number of cycles before the fluorescence intensity of the reaction reached the preset threshold, which means at that time amplification was in log phase).

CCK-8 assay

When transfected reached approximately 80% confluence, MG-63 cells were rinsed with PBS twice and digested into a single-cell suspension with 0.25% trypsin. After they were counted, the cells were seeded in a 96-well plate at a density of $3 \sim 6 \times 10^3$ cells per well in a volume of 200 µl per well. Each condition was

repeated in 6 wells. After the cells were cultured for 24 h, 48 h and 72 h, the plate was removed from the incubator, and 10 μ l of CCK8 was added per well. Then, the cells were cultured for another 2 h. The optical density (OD) of each well was detected at 450 nm using an enzyme-linked immunosorbent assay (ELISA). Every experiment was repeated 3 times. A cell survival curve was drawn with time on the abscissa and OD on the ordinate.

Cell Doubling Test

When density of MG-63 cells reached approximately 80%, the cells were trypsinized with 0.25% trypsin and then made into a single-cell suspension. After counting, the suspension was seeded in a 6-well plate with 2×10^3 cells per well and a volume of 200 μ l per well. A total of 6 replicates in each group were set. In addition, the cells were cultured in incubator for 7 days to digest and count. Doubling time = $[\lg 2 / (\lg N_0 - \lg N_7)] \times 24$ (N0 indicates the cell number at day 0; N7 indicates the cell number at day 7). The experiment was repeated 3 times.

Colony formation assay

Cells cultivated on an LB plate were digested, centrifuged, counted and re-suspended in 1640 medium. Then, the cells were seeded at a concentration of 500 cells per culture dish (10 cm) and cultured for 2 weeks at 37 °C in an incubator containing 5% CO₂. After removal of the medium, the cells were rinsed 3 times with PBS, fixed with 4% paraformaldehyde at room temperature for 20 min and stained with 0.1% crystal violet solution for 60 min. The stain was slowly removed. When the dishes were air-dried, the number of clones among more than 50 cells was counted under a microscope. The experiment was repeated 3 times.

Scratch test

After transfection for 48 h, MG-63 cells from all the groups were collected and seeded into a 6-well plate at a density of 1×10^5 cells per well to approximately 90% confluence. Four horizontal and four vertical scratches were made using a 200- μ l pipette tip. The width of several scratches were measured. The healing rate of a scratch was calculated as follows: $\{(\text{scratch width at 0 h} - \text{scratch width at 24 h}) / \text{scratch width at 0 h}\} \times 100\%$. The assay was conducted 3 times to compare the migration of the cells in three groups.

Transwell assay

Matrigel (Corning, USA) was thawed overnight at 4 °C and diluted with serum-free 1640 medium (1:3). A total volume of 30 μ l of diluted Matrigel was added to the upper chamber of every transwell chamber 3 times (15 μ l, 7.5 μ l, 7.5 μ l) with 10-min incubation steps between each addition to evenly cover all the micro-wells in the bottom of the chamber. At 48 h after transfection, MG-63 cells from all the groups were suspended and added in the upper chamber of the Transwell (Corning, USA). A total of 0.5 ml of 1640 medium containing 10% FBS was added to the lower chamber of a 24-well plate. After 48 h of culture at 37 °C in an atmosphere containing 5% CO₂, non-migratory cells in the upper chamber were carefully removed. The membranes were soaked in 95% ethanol for 15 to 20 min and stained with a crystal violet solution for 10 min. After the inserts were rinsed, cells were counted and photographed at high magnification using an inverted microscope. The collected data were the means of the number of cells under 5 randomly selected fields of vision. The number of cells that migrated through the Matrigel was regarded as the evaluation index of cell migration. The experiment was repeated 3 times.

Flow cytometry with Annexin V/PI double staining

After transfection for 48 h, MG-63 cells were digested with trypsin without ethylenediamine tetraacetic acid (EDTA), collected in the flow tube (both of supernatant and solution contained a few dead cells and apoptotic cells, and all these were included in), and centrifuged at 1000 r/min for 5 min. Then, the supernatant was removed. Cells were rinsed with ice-cold PBS 3 times and centrifuged before the supernatant was discarded. In accordance with the Annexin-V-FITC apoptosis determination kit (Sigma, St. Louis, Missouri, USA), every tube of cells was added to 150 μ l Binding buffer and 5 μ l Annexin-V-FITC and evenly mixed. After incubation in the dark at room temperature for 15 min, another 100 μ l of Binding buffer and 5 μ l PI stain (Sigma, St. Louis, Missouri, USA) was added to each tube, which was then shaken. The negative control group did not receive Annexin V treatment. Cell apoptosis was detected using flow cytometry (Coulter Corporation Co., Ltd., California, USA) within 1 hour of staining as soon as possible. The

results of the flow cytometry experiments were displayed as 4 quadrants, in which the upper right quadrant indicated advanced apoptosis rate; the lower right quadrant represents the apoptosis rate during the early stages; the upper left quadrant represents dead cells; and the lower left quadrant represents living cells. The apoptotic rate was calculated as follows: apoptotic rate of cells in the advanced stage + the apoptotic rate of cells in early stage. This experiment was repeated 3 times.

Flow cytometry with PI staining

After transfection for 48 h, MG-63 cells in all the groups were collected and rinsed with ice-cold PBS 3 times and then centrifuged before the supernatant was removed. The cell suspension was adjusted to approximately 1×10^5 cells/ml. Cells were fixed in 1 ml of ice-cold 75% ethanol at 4 °C overnight. Before staining, cells were rinsed with PBS twice, and the supernatant was removed. The cells were stained with 100 µl RNaseA and immersed in a 37 °C water bath for 30 min in the dark followed by staining with 100 µl of PI and incubating the mixture at 4 °C for 30 min in the dark. Flow cytometry (Coulter Corporation, Beckman Coulter, California, USA) was used to detect the red fluorescence at 488 nm to identify the phase of the cell cycle. The experiment was repeated 3 times.

Statistical analysis

SPSS 21.0 software (SPSS Inc., Chicago, IL, USA) was applied for statistical analysis. The measurement data were expressed as the mean $\pm$ standard deviation (SD). Comparisons between two groups were assessed using a *t* test (either the independent sample *t* test or paired *t* test). One-way analysis of variance (ANOVA) was used to compare multiple groups. $P < 0.05$ was considered statistically significant.

Results

Expression of lnc-HOST2 in the osteosarcoma tissues and adjacent normal tissues

The results indicated that compared with the adjacent normal tissues, the expression of lnc-HOST2 in the osteosarcoma tissues was significantly increased ($P < 0.05$) (Fig. 1). The correlation between the expression of lnc-HOST2 and the clinicopathological characteristics of osteosarcoma patients was analyzed. Comparisons between two groups were conducted using a *t* test and among multiple groups using an *F* test. As shown in Table 1, there was no significant correlation between the expression of lnc-HOST2 and age, gender, or histopathologic subtype of osteosarcoma among the patients (all $P > 0.05$). The above results suggested that lnc-HOST2 was highly expressed in the osteosarcoma tissues; therefore, a close correlation might exist between the expression of lnc-HOST2 and the occurrence and development of osteosarcoma.

Expression of lnc-HOST2 in four cell lines

The osteosarcoma cell lines SaOS2, HOS, MG-63 and U2OS were cultured. qRT-PCR was performed to detect the expression of lnc-HOST2. As shown in Table 2, lnc-HOST2 was expressed in the SaOS2, HOS, MG-63 and U2OS cell lines. The expression of lnc-HOST2 was highest in the MG-63 cell line and lowest in the U2OS cell line. Thus, MG-63 cells were selected for all subsequent experiments.

Expression of lnc-HOST2 in MG-63 cells among the three groups

The expression of si-lnc-HOST2 in MG-63 cells was tested using qRT-PCR. The results showed that after transfection with si-lnc-HOST2, the expression of lnc-HOST2 in this MG-63 cell line was significantly decreased compared with

Fig. 1. Expression of lnc-HOST2 in osteosarcoma and normal adjacent tissues. * refers to $P < 0.05$ compared with adjacent normal tissues.

that in the blank and si-CON groups (both $P < 0.05$). There was no significant difference between the blank group and si-CON group (both $P > 0.05$) (Fig. 3).

Effect of lnc-HOST2 on the proliferation of MG-63 cells among the three groups

The CCK8 assay was conducted to detect the proliferation of MG-63 cells after knocking down the expression of lnc-HOST2. The OD value (which reflects the proliferation rate) was calculated, and proliferation curves at 24 hours, 48 hours and 72 hours were constructed. As shown in the results of the CCK8 assay (Fig. 4A), the MG-63 cells proliferation in the si-lnc-HOST2 group was obviously slower down. Compared with the blank group and the si-CON group, the si-lnc-HOST2 group had significantly decreased OD values at 48 h and 72 h. The proliferation of MG-63 cells was significantly inhibited when lnc-HOST2 expression was inhibited (both $P < 0.05$). There was no significant difference in cell proliferation between the blank group and si-CON group ($P > 0.05$). The results of the doubling time experiment indicated that the si-lnc-HOST2 group (36.04 ± 0.79) showed a longer doubling time than the si-CON group (23.04 ± 0.33) and the blank group (22.85 ± 0.21) ($P < 0.05$) (Fig. 4B). It was shown that the si-lnc-HOST2 group had a higher cell number than the blank and si-CON groups over the same duration of culturing (48 or 72 h), which indicated that si-lnc-HOST2 slowed the growth of MG-63 cells. The results mentioned above indicate that down-regulating lnc-HOST2 inhibited the proliferation of MG-63 cells.

Effect of lnc-HOST2 on colony formation among the three groups

The results of the colony formation assay were as follows. The number of MG-63 cell colonies in the si-lnc-HOST2 group was significantly decreased compared with the numbers in the blank group and si-CON group ($P < 0.05$), while there was no significant difference between the blank group and si-CON group ($P > 0.05$). The results suggested that down-regulation of lnc-HOST2 inhibited the growth of MG-63 cells (Fig. 5).

Table 1. Correlations between the relative expression of lncRNA-HOST2 and clinicopathological characteristics of patients with osteosarcoma. lncRNA-HOST2, long non-coding RNA-HOST2; t, the test value of independent sample t test; F, the test value of one-way analysis of variance

Characteristic	n	lncRNA-HOST2 expression	t/F	P
Age (years)			1.076	0.295
< 15	20	7.11 ± 1.49		
≥ 15	32	6.69 ± 1.29		
Histological type			1.86	0.170
Osteogenic tissue	25	7.21 ± 1.63		
Cartilaginous tissue	16	6.64 ± 1.05		
Fibrous tissue	11	6.35 ± 1.05		
Gender			1.978	0.053
Male	25	6.69 ± 1.29		
Female	27	7.11 ± 1.49

Fig. 2. lnc-HOST2 expressions in the SaOS2, HOS, MG-63 and U2OS cell lines.

Fig. 3. Expression of lnc-HOST2 in MG-63 cells among the three groups after transfection for 48h. * refers to $P < 0.05$ compared with the blank group and si-CON group.

Fig. 4. Cell proliferation (A) and doubling time (B) (as detected using the CCK-8 assay) of MG-63 cells among the three transfected groups. * refers to $P < 0.05$ versus the blank group and si-CON group.

Fig. 5. Cell growth (A) and colony number (B) of MG-63 cells as detected using the colony formation assay. * refers to $P < 0.05$ compared with the blank group and si-CON group.

Effect of lnc-HOST2 on the migration of MG-63 cells among the three groups

As shown in Fig. 6, after 48 h of cultivation, the healing rates after scratching the MG-63 cell monolayers in the blank group and si-CON group were $(73.56 \pm 2.43)\%$ and $(71.05 \pm 3.69)\%$, respectively ($P > 0.05$). The healing rate of scratch of MG-63 cells in the si-lnc-HOST2 group was $(51.84 \pm 2.57)\%$, which was significantly lower than that in the blank group and si-CON group ($P < 0.05$). The results indicated that down-regulation of lnc-HOST2 inhibited the migration of MG-63 cells.

Effect of lnc-HOST2 on the invasion of MG-63 cells among the three groups

The number of cells migrating to the bottom of the Transwell through the Matrigel in the blank group and si-CON group was 54.67 ± 14.23 and 51.92 ± 10.45 , respectively ($P > 0.05$). The number of cells that migrated to the bottom of the Transwell through the Matrigel in the si-lnc-HOST2 group was 35.64 ± 13.86 , which was significantly lower than that in the blank group and si-CON group (both $P < 0.05$). The results showed that down-regulation of lnc-HOST2 inhibited the invasion of MG-63 cells (Fig. 7).

Effect of lnc-HOST2 on the apoptosis of MG-63 cells among the three groups

Flow cytometry of PI-stained cells was used to detect the cell cycle distribution of MG-63 cells when lnc-HOST expression was knocked down, and the results were as follows: the proportion of MG-63 cells in G1 phase among the blank group, si-CON group and si-lnc-HOST2 group were $(50.35 \pm 1.74)\%$, $(51.42 \pm 1.16)\%$ and $(67.23 \pm 1.26)\%$, respectively (Fig. 8). In the si-lnc-HOST2 group, cell cycle was primarily halted at G1 phase, while the proportion of the cells in S phase decreased. The proliferation of MG-63 cells with lnc-HOST knockdown was inhibited compared with that in the blank group and si-CON group ($P < 0.05$). There was no significant difference between the blank group and si-CON group ($P > 0.05$).

Fig. 6. Cell migrations of MG-63 cells by scratch test ($\times 40$ magnification). A: Blank group (no transfection); B: si-CON group (transfected with negative control siRNA); C: si-lnc-HOST2 group (transfected with small interfering lnc-HOST2 siRNA); D: the healing rate of MG-63 cells in the three groups after transfection and assessed using the wound-scratch assay. * refers to $P < 0.05$ compared with the blank group and si-CON group.

Fig. 7. Cell invasion of MG-63 cells as assessed using the Transwell assay ($\times 200$). A: Blank group (no transfection); B: si-CON group (transfected with negative control siRNA); C: si-lnc-HOST2 group (transfected with small interfering lnc-HOST2 siRNA); D: the number of the invasive cells in the three groups after transfection. * refers to $P < 0.05$ compared with the blank group and si-CON group.

Fig. 8. Cell cycle distribution of MG-63 cells among the three groups after a 48-h of transfection and assessment using flow cytometry with PI staining. A: Cell cycle distribution of MG-63 cells in the three groups after transfection. B: proportion of MG-63 cells in G1, G2, and S phases among the three transfected groups. * refers to $P < 0.05$ compared with the blank group and si-CON group.

Flow cytometry with Annexin V/PI double staining was used to assess the number of apoptotic MG-63 cells after knocking down lnc-HOST expression, and the results were as follows: the apoptosis rates of the blank group, si-CON group and si-lnc-HOST2 group after 48 h of transfection were $(17.54 \pm 0.67)\%$, $(18.65 \pm 0.86)\%$ and $(26.75 \pm 1.84)\%$, respectively. Compared with the blank group and si-CON group, the si-lnc-HOST2 group exhibited a significantly increased apoptosis rate of MG-63 cells ($P < 0.05$). There was no

Fig. 9. Apoptosis of MG-63 cells among the three groups 48 h after transfection as assessed using flow cytometry with Annexin V/PI double staining. A: The apoptosis of MG-63 cells in the three groups after transfection. B: apoptosis rates of MG-63 cells in the three groups after transfection. $P < 0.05$ compared with the blank group and si-CON group.

significant difference between the blank group and si-CON group regarding the apoptosis rate ($P > 0.05$) (Fig. 9). The results described above revealed that down-regulation of lnc-HOST2 significantly promoted the apoptosis of MG-63 cells.

Discussion

Osteosarcoma is one of the most common primary malignant bone tumors in children. Treatment methods such as chemotherapy and limb-sparing surgery have been applied for osteosarcoma [17]. However, these treatment methods induce increased risk of relapse and the development of metastasis in some patients [12]. Understanding the mechanisms and underlying pathogenesis of osteosarcoma may help yield novel biomarkers for early detection and treatment. It has been indicated that lncRNAs are related to the occurrence and development of many human diseases and may play potential roles in physiological processes [18, 19]. In addition, previous studies have revealed the presence of differentially expressed lncRNAs in osteosarcoma and suggest that lncRNAs may be novel candidate biomarkers for the diagnosis of osteosarcoma [20, 21]. lncRNAs have been implicated in many cellular processes and the dysregulation of lncRNAs could play an important role in osteosarcoma [22]. The aim of this study was to determine the effects of lnc-HOST2 on the proliferation, migration, invasion and apoptosis of osteosarcoma cells.

In our study, the expression of lnc-HOST2 was observed in both osteosarcoma cell lines and clinical specimens. The expression of lnc-HOST2 in osteosarcoma tissues was significantly higher than that in adjacent normal tissues. Our data indicated that lnc-HOST2 could promote the proliferation of osteosarcoma cells relative to osteosarcoma progression. Wang *et al.* [23] indicated that the expression of lncRNAs such as MEG3 was significantly decreased in osteosarcoma, which suggests that lncRNAs might function as tumor suppressors [23]. For these reasons, we hypothesized that lnc-HOST2 could be a promising biomarker and therapeutic target for the treatment of osteosarcoma.

Furthermore, to study the effect of lnc-HOST2 on biological progression of osteosarcoma, we observed the proliferation, migration and invasion of MG-63 cells after inhibiting lnc-HOST2 expression. The results showed that down-regulation of lnc-HOST2 could effectively inhibit the proliferation, migration and invasion as well as promote apoptosis of osteosarcoma cells. lnc-HOST2 has been reported to be highly expressed in human cancers, and lnc-HOST2 inhibits let-7b function, which could post-transcriptionally inhibit the expression of targets such as some oncogenes that regulate cell growth and motility [14]. In addition, HMGA2, c-Myc, Dicer and Imp3, all of which are targets of lnc-HOST2, appear to participate in cell cycle regulation and malignant transformation; however, HMGA2 has been primarily implicated in the pathogenesis of benign, mesenchymal tumors [24]. c-Myc is the most common oncogene involved in the regulation of cell cycle and promotes cell apoptosis [25]. Dicer is known as

a central enzyme in microRNA processing and is important to produce mature microRNAs, which would regulate post-transcriptional gene expression [26]. Overexpression of lmp3 in calvarial osteoblasts, dermal fibroblasts, and bone marrow stromal stem cells stimulates osteo lineage differentiation *in vitro* [27]. For these reasons, we suggested that lnc-HOST2 could be a control factor not only in ovarian cancer but also in osteosarcoma cells; additionally, the miRNA Let-7b plays a role in transcriptional regulation. Thus, the expression of HMGA2 and c-Myc as well as the proliferation, invasion and migration of tumor cells are promoted. Understanding lnc-HOST2/let-7b-dependent regulation may lead to alternative approach for the diagnosis and cure of osteosarcoma. However, the concrete mechanism of lnc-HOST2 still needs further study.

Conclusion

Taken together, lnc-HOST2 could be highly expressed specifically in osteosarcoma and participate in regulating the migration, invasion and proliferation of osteosarcoma cells. These processes might be closely associated with the development of malignant tumors. Thus, lnc-HOST2 could be a new potential target for the treatment of osteosarcoma. However, the specific mechanism of how lnc-HOST2 functions in osteosarcoma is still stalled due to a lack of adequate experimental data. Thus, the next step is to construct recombinant lnc-HOST2 plasmid to increase the levels of endogenous expression and study the mechanism of biological function of osteosarcoma cells in depth.

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Disclosure Statement

Authors declare that they have no existing conflicts of interest concerning this article.

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